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The Moldovan Medical Journal is an international scientific double-blind peer reviewed periodical edition, 4 per year, of the Scientific Medical Association of the Republic of Moldova designed for specialists in the areas of medicine, dentistry, pharmacy, social medicine and public health. From its debut the journal has striven to support the interests of Moldovan medicine concerning the new concepts of its development.

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TABLE OF CONTENTS

ORIGINAL RESEARCHES

Antimicrobial susceptibility and biofilm production among *Staphylococcus* and *Candida* species 3-7
Greta Balan, Olga Burduniuc

Combined therapeutic approach in acute coronary syndrome patients under environmentally unfriendly working conditions 8-12
Mamedgasan Agaev, Tarana Agaeva

Automatic amperometric titration method for quantitative determination of zinc oxide in ointments....13-18
Vasile Oprea, Vladimir Valica, Constantin Cheptanaru, Mihai Nistorica, Serghei Oprea, Liviu Movila

Estimating the clinical needs for tissues and cells in the Republic of Moldova....19-25
Tatiana Timbalari

Prevalence of overweight in adults in the Republic of Moldova 26-28
Angela Tomacinschii, Oleg Lozan

Umbilical cord coiling abnormality as a predictor of maternal and fetal outcomes 29-32
Alina Alsatou

Heart rate variability in people with borderline type personality....33-38
Svetlana Lozovanu, Ion Moldovanu, Victor Vovc, Tudor Besleaga, Andrei Ganenco

REVIEW ARTICLES

Molecular characterization of the endometrium as a fertility-determining factor....39-43
Mihaela Burac, Natalia Corolcova, Valentin Friptu

Transcatheter aortic valve implantation – new era in treatment of aortic stenosis....44-51
Oleg Calenici

Acupoint embedding therapy....52-58
Olga Ignatov, Oleg Pascal, Viorel Nacu

General provisions on medication errors committed by pharmacists 59-63
Nicoleta Cheptanari-Birta

Trauma scoring systems....64-74
Oleg Arnaut, Dan Croitoru, Ion Grabovschi, Serghei Sandru

BOOK REVIEW

Mihail Maniuc
The Monograph "Evolution of otitis media in children". The author: **Svetlana Diakova**....75

GUIDE FOR AUTHORS....76

Editorial Staff

Ludmila Martinenko, English Corrector, telephone: +373 22 205 209
Irina Litvinenko, Editorial Secretary, telephone: +373 32205877

Address of the Editorial Office
192, Stefan cel Mare Avenue, Chisinau, MD-2004, the Republic of Moldova
Phone: +373 22 205 209, Office: +373 79 429 274 mobile
www.moldmedjournal.md editor@moldmedjournal.md

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ORIGINAL RESEARCHES

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Antimicrobial susceptibility and biofilm production among *Staphylococcus* and *Candida* species

*[1,2]Greta Balan, [1,2]Olga Burduniuc

[1]Department of Microbiology and Immunology, *Nicolae Testemitsanu* State University of Medicine and Pharmacy
[2]Department of Laboratory Diagnostic in Public Health, National Agency for Public Health
Chisinau, the Republic of Moldova

Authors' ORCID iDs, academic degrees and contributions are available at the end of the article

*Corresponding author: greta.balan@gmail.com
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Abstract

Background: Biofilms are surface-attached groups of microbial cells that are embedded in an extracellular matrix. One of the main features of biofilms is their resistance to antimicrobial drugs; therefore, the biofilm-based infections are extremely difficult to treat. This study aimed to investigate the biofilm-forming capacity of *Staphylococcus* spp. and *Candida* spp. strains isolated from collected clinical samples, as well as to assess their antibiotic susceptibility.
Material and methods: The study was conducted on 134 strains of *Staphylococcus* spp. and 147 strains of *Candida* spp. isolated from various clinical specimens. Both biofilm formation and antibiotic susceptibility of the isolated strains were studied using contemporary standardized microbiological methods.
Results: The results of the study showed a high biofilm-forming capacity among the clinical strains of *Staphylococcus* spp. and *Candida* spp., as well as a higher level of antibiotic resistance in biofilm-producing strains compared to biofilm non-producing ones.
Conclusions: The high rates of antibiotic resistance and biofilm-forming capacity of strains represent a major public health challenge. The study showed a strong correlation between biofilm formation and antimicrobial resistance patterns.
Key words: *Staphylococcus* spp., *Candida* spp., biofilm formation, antimicrobial resistance.

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Introduction

The advancement of biomedical science has enabled to study the microorganisms in their natural environment, whereas over 95% of microorganisms existing in nature are in biofilms [1]. Biofilm formation is an important strategy by which microorganisms survive and adapt in natural environments [2, 3].

A biofilm is defined as an aggregate of microorganisms in which the cells adhere to each other on a surface, enclosed in a synthetized extracellular polymeric substance matrix. Biofilms can occur on living or non-living surfaces, being widely spread in nature. The vast majority of bacterial infections may also involve microbial biofilm formation [4].

Bacteria living in a biofilm usually have significantly different properties from free-floating bacteria of the same species, being protected by a dense biofilm structure, which allows them to cooperate and interact in different manners. The main features of the biofilms are their high resistance to disinfectants and antimicrobial drugs; whereas the thick extracellular matrix and the outer layer cells protect the interior of the community [5].

Most microorganisms form biofilms as a means of response to a number of factors, including cellular recognition of specific or non-specific attachment sites on a surface nutritional index, or in some cases, by exposure of planktonic cells to sub-inhibitory concentrations of antibiotics [6, 7].

It is estimated that microbial biofilms play a major role in over 80% of infections. Sixty percent of healthcare-associated infections are due to biofilm formation on medical implants. Moreover, many chronic diseases are associated with biofilms, such as infectious endocarditis, cystic fibrosis pneumonia, periodontitis, chronic rhinosinusitis, trophic ulcers and otitis media [8].

Staphylococci, predominantly *Staphylococcus aureus* and *Staphylococcus epidermidis*, are the disease-causing agents in a series of infections, which are often associated with chronicity, difficulty to eradicate and antimicrobial resistance [9]. Staphylococci are ranked first among the etiological fac-

tors of bacterial infections, along with the annual increase in the number of methicillin-resistant staphylococci (MRS) strains and the occurrence of new antibiotic-resistant bacterial strains, which place this pathology among the emerging infectious diseases [10].

Staphylococcus aureus is an opportunistic pathogen, commonly involved in skin and soft tissue infections. It could be detected in the nasopharynx, skin, eyes, intestine and urogenital tract as part of the normal flora; although in some cases, it might pass through the skin barriers of wounds or surgical incisions, causing infections. In addition, it has the property to adhere and form biofilms on tissues or medical devices. Coagulase-negative staphylococci (CoNS) are considered saprophytic, avirulent or low-virulent microorganisms. However, over the past three decades there has been an increase in human infections caused by CoNS, particularly of *S.epidermidis* [11].

Levuriform fungi of the genus *Candida* are found as part of the normal flora in healthy individuals and are involved in the etiology of opportunistic infections, resulting in high mortality rates, particularly in immunocompromised individuals [12]. *Candida* species are most commonly associated with human diseases due to both virulence factors and biofilm-forming ability. *Candida* spp. causes systemic diseases and is the fourth most common cause of hospital-acquired blood infections. *Candida albicans* is the most commonly found species in fungal infections, whereas other species are involved to a lesser extent. However, the increased rate of non-Candida albicans isolation and antimicrobial resistance has become a major challenge for clinicians over the recent years [13].

Most infections caused by *Candida* spp. are related to biofilm formation on the mucosal surfaces and contaminated medical devices. Some study results revealed that the biofilms, formed by *Candida* spp. may become resistant to antifungal drugs, including amphotericin B, fluconazole, flucytosine, itraconazole and ketoconazole [14].

Therefore, a current *in vitro* study of the biofilm-forming ability associated with the antimicrobial resistance patterns of *Candida* spp. and *Staphylococcus* spp. strains isolated from various clinical biosubstrates is required for the efficient management of these infections.

Material and methods

There have been examined 134 strains of *Staphylococcus* spp. (88 – *S. aureus,* 46 – *S. epidermidis*) and 147 strains of *Candida* spp. (75 – *C. albicans,* 24 – *C. glabrata,* 22 – *C. krusei,* 14 – *C. parapsilosis,* 12 – *C. tropicalis*), isolated from clinical biosubstrates (blood, trophic ulcers, infected wounds, and vaginal secretions) and which have been identified by standard microbiological techniques [15].

Antimicrobial susceptibility testing and the result interpretation were carried out according to EUCAST (The European Committee on Antimicrobial Susceptibility Testing) by using both qualitative methods (Kirby-Bauer disk diffusion assay) and quantitative methods determining the minimum inhibitory concentration (E-test, Vitek 2 Compact) [16].

Staphylococcus spp. strains were tested for benzylpenicillin, gentamicin, norfloxacin, cefoxitin, chloramphenicol, erythromycin, clindamycin, tetracycline, rifampicin, linezolid and vancomycin, whereas *Candida* spp. strains were assessed to fluconazole, itraconazole, amphotericin B, micafungin and flucytosine.

Bacteria that showed resistance to at least one preparation out of three or more antimicrobial groups were identified as multidrug resistant strains (MDR) in accordance with the guidelines recommended by the joint initiative of the European Center for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC) [17]. The methicillin-resistant or methicillin-sensitive (MSS) patterns of *Staphylococcus* spp. strains were determined according to the inhibition zone diameters of cefoxitin disk (30mg), based on EUCAST: MSS if the diameter is at least 22 mm; MRS if less than 22 mm. A double disc diffusion test (D test) was used for detecting inducible resistance to clindamycin. The erythromycin (15mg) and clindamycin (2mg) discs are placed at a distance of 12-20 mm measured from the edges of the discs. A flattening of the zone of inhibition around the clindamycin disk (D test positive) is reported as a clindamycin-resistance [18].

Biofilm production by isolated strains was quantitatively determined using the microtiter plate method [19]. For the purpose of study, 150µl of peptonate broth and 15µl of bacterial suspension were added to a 96 well plate and adjusted to the 0.5 McFarland turbidity standard (respectively $1.5x10^8$ CFU/ml), which were previously prepared from 18-24 hour bacterial culture and grown on 5% blood agar. The plates were coated and incubated for 24 hours at 37° C. Subsequently, the level of adhesion of the tested strains to inert substrate was determined by removing the content from each well and then rinsing five times with sterile saline and fixing with cold methanol for 5 minutes. After removing of the methanol, the dried plates were stained with 0.1% violet crystal solution for 30 minutes. The excess stain was removed by washing and the stained biofilm was resuspended in a 33% glacial acetic acid solution. Thus the obtained suspensions were used to determine the optical density (OD), based on the spectrophotometric absorbance readings at 570 nm colored suspension (A570). The tests were performed in duplicate.

The optical density cut-off value (ODc) is defined as the average OD of negative control + 3x the standard deviation (SD) of negative control. Biofilm formation by the tested strains was assayed and classified according to the adsorption of the violet crystal dye. The isolates were classified into four categories: non-adherent (OD ≤ ODc), poor adherent (ODc <OD ≤ 2xODc), moderately adherent (2xODc <OD ≤4xODc) and strongly adherent (4xODc <OD).

The reference strains *Staphylococcus aureus* (ATCC 25923), *Candida albicans* (ATCC 10231) and *Candida tropicalis* (ATCC 750) were used for quality control. EpiInfo 2000 was used in statistical data analysis.

Results

The antimicrobial susceptibility testing results of 134 strains of *Staphylococcus* spp., revealed that 92 (68.6%) were polyresistant to antibiotics, 69 (51.5%) were methicillin-resistant, and 32 (23.9%) were D-test positive.

Staphylococcus spp. strains showed the highest sensitivity levels to vancomycin (100%), followed by tetracycline (88.8%), linezolid (83.6%) and chloramphenicol (82.8%) (fig. 1).

Invasive candidiasis is usually treated with five main groups of antifungal drugs, including azoles, polyenes, allylamines, echinocandins and pyrimidine analogues [20]. A study, conducting a susceptibility testing for *Candida* species to fluconazole, voriconazole, itraconazole, ketoconazole and flucytosine, showed that most *Candida* spp. strains were sensitive to fluconazole and flucytosine [21].

The studied *Candida* spp. strains showed different levels of susceptibility to the tested antimycotics. The data analysis showed the highest level of resistance to itraconazole (87.7%) and fluconazole (87.1%), followed by amphotericin B (10.9%) and micafungin (2.7%). All tested strains were found to be sensitive to flucytosine (fig. 2).

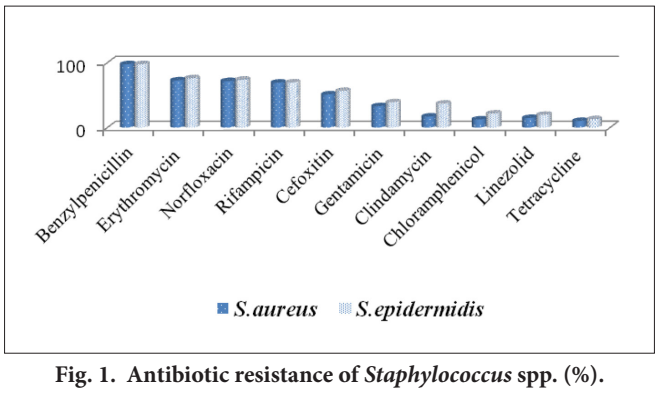

Fig. 1. Antibiotic resistance of *Staphylococcus* spp. (%).

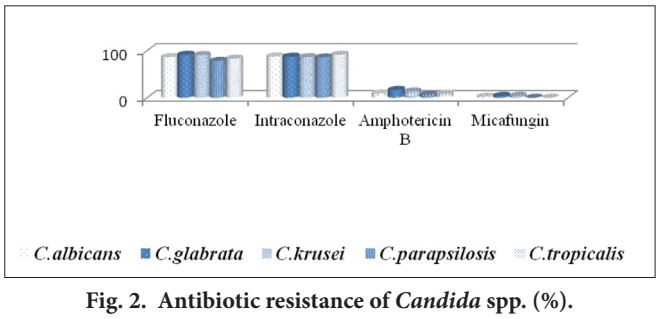

Fig. 2. Antibiotic resistance of *Candida* spp. (%).

The next stage of the study determined the biofilm formation ability of *Staphylococcus* spp. and *Candida* spp. Of the 134 tested staphylococcus strains, 77 (57.5%) produced detectable biofilms. The biofilm status referred to 27 (35.1%) of isolates, which produced strong biofilms, 32 (41.6%) – moderate biofilms and 18 (23.4%) – weak biofilms (fig. 3).

Candida spp. strains produced detectable biofilms in 59.2%. The highest level of biofilm formation ability was recorded in *C.glabrata* strains (95.8%), followed by *C. parapsilosis* (57.1%), *C. krusei* (54.5%), *C. albicans* (52.0%) and

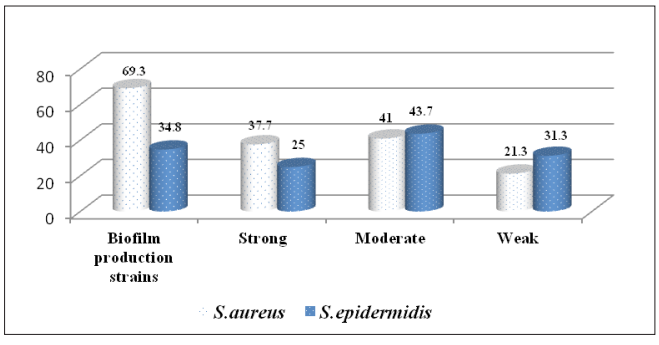

Fig. 3. The biofilm formation capacity of *Staphylococcus* spp. (%).

C. tropicalis (41.7%). 44 (50.6%) of *Candida spp*. strains produced strong biofilms, 29 (33.3%) – moderate biofilms and 14 (16.1%) – weak biofilms (fig. 4).

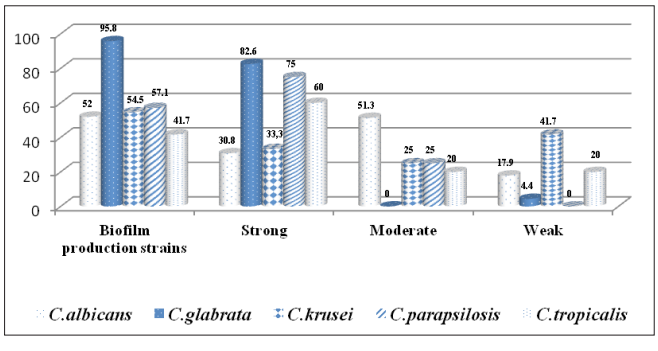

Fig. 4. Biofilm formation capacity of *Candida* spp. (%).

It is a well-known fact that bacterial populations in biofilms are considerably more resistant to antibiotics than planktonic cells [22]. Thus, biofilm-producing *Staphylococcus* spp. strains showed a higher antibiotic resistance compared to non-producing strains: benzylpenicillin (100% vs. 94.7%), gentamicin (61.0% vs. 0%), erythromycin (94.8 % vs 45.6%), tetracycline (19.5% vs 0%), cefoxitin (68.8% vs 42.1%), clindamycin (38.9 vs 3.5%), norfloxacin (90.9% vs 47.4%), chloramphenicol (27.3% vs 0%), rifampicin (81.7% vs 29.8%) and linezolid (25.9% vs 3.5%). All strains were found sensitive to vancomycin (tab. 1).

Comparison of biofilm formation ability between methicillin-resistant (MRS) and methicillin-sensitive (MSS) isolates of *Staphylococcus* spp. was carried out. The quantitative and qualitative results showed higher biofilm formation ability in MRS strains for both *S.aureus* and *S. epidermidis* strains compared to MSS bacteria. Biofilm-producing strains revealed a higher antibiotic resistance, which may lead to treatment failures in MRS infections (tab. 2).

The studies on *Candida* spp. strain resistance to antifungal drugs, as well as biofilm formation capacity, showed a statistical correlation between biofilm formation capacity and antifungal susceptibility ($p < 0.05$) (tab. 3).

Flucytosine is known to inhibit both ribonucleic acid and deoxyribonucleic acid synthesis [23] and was the most effective antifungal agent against biofilm-producing *Candida* strains, tested within the present study.

Table 1

Antibiotic resistance of biofilm-producing and non-producing *Staphylococcus* spp.

Antimicrobials	Biofilm-producing strains (N=77)	Biofilm-nonproducing strains (N=57)	p-value
	n (%)	n (%)	
Penicillins			
Benzylpenicillin	77 (100)	54 (94.7)	$p>0.05$
Aminoglycosides			
Gentamicin	47 (61.0)	0 (0)	$p<0.0001$*
Macrolides			
Erythromycin	73 (94.8)	26 (45.6)	$p<0.0001$*
Tetracyclines			
Tetracycline	15 (19.5)	0 (0)	$p>0.05$
Cephalosporins			
Cefoxitin	53 (68.8)	24 (42.1)	$p<0.05$*
Lincosamides			
Clindamycin	30 (38.9)	2 (3.5)	$p>0.05$
Fluoroquinolones			
Norfloxacin	70 (90.9)	27 (47.4)	$p<0.0001$*
Miscellaneous agents			
Chloramphenicol	21 (27.3)	0 (0)	$p<0.05$*
Rifampicin	76 (81.7)	17 (29.8)	$p<0.0001$*
Oxazolidinones			
Linezolid	20 (25.9)	2 (3.5)	$p>0.05$
Glycopeptides			
Vancomycin	0 (0)	0 (0)	NA

Note: *Statistically significant ($p<0.05$); NA – not applicable.

Table 2

Biofilm formation capacity of MRS and MSS *Staphylococcus* spp.

Biofilm production	S.aureus			S.epidermidis		
	MRSA n (%)	MSSA n (%)	Total n (%)	MRSE n (%)	MSSE n (%)	Total n (%)
Strong	16 (26.2)	7 (11.5)	23 (37.7)	4 (25.0)	0 (0)	4 (25.0)
Moderate	17 (27.9)	8 (13.1)	25 (41.0)	6 (37.5)	1 (6.3)	7 (43.7)
Weak	7 (11.5)	6 (9.8)	13 (21.3)	3 (18.7)	2 (12.5)	5 (31.3)

Note: MRSA – methicillin resistant *S. aureus*; MSSA – methicillin sensitive *S. aureus*; MRSE – methicillin resistant *S. epidermidis*; MSSE – methicillin sensitive *S. epidermidis*.

Table 3

Antimicrobial resistance of biofilm- producing and non-producing *Candida* spp. strains

Antimicrobials n (%)		Biofilm-producing strains (N=87)	Biofilm-nonproducing strains (N=60)	p-value
		n (%)		
Azoles				
Fluconazole	87 (100)	41 (68.3)	$p<0.0001$*	
Intraconazole	87 (100)	42 (70.0)	$p<0.0001$*	
Polyenes				
Amphotericin B	15 (17.2)	1 (1.7)	NA	
Echinocandins				
Micafungin	4 (4.6)	0 (0)	$p>0.05$	
Pyrimidine analogue				
Flucytosine	0 (0)	0 (0)	NA	

Note: *Statistically significant ($p<0.05$); NA – not applicable.

Conclusions

The study results revealed a higher biofilm formation capacity in the clinical strains of *Staphylococcus* spp. and *Candida* spp. as well as higher rates of antimicrobial resistance in biofilm-producing strains compared to non-producing ones. The obtained data proves a strong correlation between biofilm formation capacity and antimicrobial resistance patterns. The implementation of the relevant antimicrobial susceptibility testing of biofilm-producing strains will improve the management of infections caused by these microorganisms, as well as provide feasible strategies to prevent their spread.

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Authors' ORCID iDs and academic degrees
Greta Balan – https://orcid.org/0000-0003-3704-3584, MD, MPH, PhD.
Olga Burduniuc – https://orcid.org/0000-0002-6944-0800, MD, MPH, PhD.

Authors' contributions
GB designed the trial and interpreted the data. OB revised the manuscript critically.
Both authors revised and approved the final version of the manuscript.

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The trial was authors' initiative.
The authors are independent and take responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate
The strains used in this study were obtained from the routine analysis of clinical specimens. Sample collection did not involve direct contact with the patient, thus, no consent was required. The study was approved by the ethics committee of *Nicolae Testemitsanu* State University of Medicine and Pharmacy from the Republic of Moldova, proceedings No 65/12.04.2017 and No 67/12.05.2017.

Conflict of Interests
No competing interests were disclosed.

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Combined therapeutic approach in acute coronary syndrome patients under environmentally unfriendly working conditions

***[1]Mamedgasan Agaev, [2]Tarana Agaeva**

*[1]Department of Cardiology, Azerbaijan Medical University, Baku, Azerbaijan
[2]*D. M. Abdulaev* Institute of Cardiology, Baku, Azerbaijan

Authors' ORCID iDs, academic degrees and contributions are available at the end of the article

*Corresponding author: dante.karelian@mail.ru

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Abstract

Background: The purpose of this study was to assess the effectiveness of the combined use of high doses of heparin, propranolol and monopril with percutaneous coronary intervention (PCI) on eco-endotoxicosis, systolic blood pressure, diastolic blood pressure, heart rate (HR), cardiodynamics and on the clinical course in acute myocardial infarction (AMI) among patients working in environmentally unfriendly conditions.

Material and methods: The study was conducted on 42 patients, aged 30 to 70 years (56.7 ± 1.20 years) with acute coronary syndrome (ACS), who were assessed for the anterior Q wave MI and ST segment elevation MI. Of 42 patients, 21 were treated with monopril, propranolol with heparin and PCI (group 1); and 21 patients underwent only PCI (group 2). The degree of eco-endotoxicosis in blood was studied in both groups, whereas the echocardiography and Doppler echocardiography were used to determine the end-systolic volume (ESV), end-diastolic volume (EDV), left ventricular ejection fraction (LV EF), local LV contractile dysfunction, local contractile dysfunction index (LCDI), restenosis via a repeated coronary angiography, echographic study of ST segment elevation and of repeated anginal pain.

Results: Patients treated with monopril with propranolol and heparin with PCI exhibited a stabilization of central hemodynamic indices, by a decrease in ESV, EDV, LCDI, and the degree of eco-endotoxicosis, as well as an improvement of LV systolic function by an increased EF. However, one patient from this group had an acute heart failure (AHF) on the 3rd day, whereas one patient experienced a MI relapse. The group of patients who underwent only PCI, revealed 3 cases of MI recurrence, 3 cases of restenosis, 2 cases of AHF and 2 patients died.

Conclusions: The combined use of drug and PCI therapy in acute coronary syndrome might lead to positive prognostic outcomes, rather than a separate PCI approach.

Key words: ecology, acute coronary syndrome, hemodynamics, percutaneous coronary intervention.

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Introduction

According to present WHO data, the high incidence rate of diseases is mainly due to the increasing rate of environmental stress: air, soil, water pollution, etc. [1-3]. The impact of anthropological and emergency factors (SO_2, H_2SO_4, NO, CO, CO_2, electromagnetic radiation, etc.) on the human body leads to chronic toxicity and eco-endotoxicosis [2-4]. Eco-endotoxicosis might increase the risks for coronary heart disease (CHD) and myocardial hypoxia, as well as reduce tissue resistance, aggravate blood and lymph rheology, and interferes with microcirculation. All these factors might result in extensive myocardial damage [1, 4], followed by heart failure (HF), abnormal heart rate (HR) and sudden coronary death in myocardial infarction (MI) [1, 3]. Therefore, new methods are currently being in search to reduce the level of average peptide molecular weight (PMW), as well as prevent or reduce the occurrence of various complications in early stages of MI [2, 3, 7, 12, 13]. Thrombolytic therapy and angioplasty (percutaneous coronary intervention – PCI) of the impaired coronary vessel have recently been used to reduce complications in the early MI stages [1, 5]. Therefore, there is a great demand in searching for new methods to prevent and decrease the occurrence of various complications in early MI [5–9]. Thrombolytic therapy and angioplasty (percutaneous coronary intervention – PCI) of the impaired coronary vessel have recently been used to reduce complications in the early MI [5, 7, 8]. However, there are evidences that every third patient develops a recurrent myocardial infarction on already the first day after the thrombolytic therapy has been carried out [5, 9, 10], followed by infarction-related coronary artery restenosis [5, 10, 11]. Moreover, the mechanical reperfusion in early myocardial infarction might increase the incidence of adverse outcomes [1, 11]. Thus, the effectiveness and safety of a combined therapeutic approach are still being discussed among specialists and there is no consensus upon this issue [5, 7, 10, 12]. At the same time, the combined use of

thrombolytics, anticoagulants, β-blockers, angiotensin converting-enzyme inhibitors (ACE inhibitors) and PCI, which play a significant role in eco-endotoxicosis among patients with acute myocardial infarction, working in ecologically unfriendly conditions is still neither fully understood nor sufficient attention is paid [1, 4, 8]. Based on the latest specialized literature data, this study was conducted on the effectiveness of a combined use of high doses of heparin, propranolol , monopril and PCI on systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), cardiodynamics and the clinical course of acute MI associated with eco-endotoxicosis, among patients exposed to ecologically unfriendly working conditions.

Material and methods

The study was conducted on 42 patients aged 30 to 70 years (56.7 ± 1.20 years), presenting with acute coronary syndrome (ACS): the anterior Q-wave myocardial infarction and ST-segment elevation acute myocardial infarction were assessed. The study included 35 (83.3%) men and 7 (33.3%) women. The patients were randomly divided into 2 groups of 21 subjects each. The 1st group was administered propraponol, heparin and monopril combined with PCI, whereas the 2nd group underwent PCI only. All patients worked in environmentally unfriendly conditions and were in contact with anthropological-emergency factors (SH_2, SO_2, H_2SO_4, NO, CO, CO_2), electromagnetic radiation, etc. (tab. 1).

Both groups were assessed for the clinical course of the disease including SBP, DBP, heart rate, the early restenosis, recurrent myocardial infarction, acute heart failure (ACF) and mortality rate during the follow-up period. Additionally, ACF was determined according to the Killip classification over the last 7 days. The echocardiographic study of heart hemodynamics was carried out via SSD-2 (Aloka. Co., Ltd. Tokyo, Japan). The end-systolic volume (ESV), end-diastolic volume(EDV), local contractile dysfunction index (LCDI), left ventricular ejection fraction (LV EF), restenosis via a repeated coronary angiography, echocardiographic assessment of ST segment elevation and of recurrent anginal chest pains were determined. Monopril was administered over the first 3 days, 2.5 mg once daily in the morning, then 5 mg / day over 10 days and 10 mg /day on the following days of MI. 5 mg of 0.1% propranolol were injected intravenously within 5 minutes, followed by a 0.02 mg / kg / min IV dosage, 20-25 drops/minute, 4 times/day. Afterwards, the patients were given a dose of 80 to 120 mg /day per oral. The 1st group of patients used heparin: 20.000 units were first administered intravenously, concomitantly with the therapeutic dose of 10.000 units subcutaneously, followed by a 6-hour administration interval on the 1st day, then 10.000 IU on the 2nd-3rd days – every 8 hours, 10.000 IU on the 4^{th}-5^{th} days – every 12 hours, 10.000 IU on the 6th day – once a day, followed by warfarin anticoagulant, 1 tablet – 2 times/ day and a daily dose of 300 mg aspirin. The patients from the 2nd group underwent PCI and were administered 300 mg of aspirin per day.

The obtained data was statistically processed by using the "Statistics 6.0" software program. The M values, their standard errors (m) and a 95% confidence interval were estimated. The study applied both the non-parametric Mann-Whitney U test criterion and the Fisher's exact test criterion. The difference was considered statistically significant with $P < 0.05$.

Results

The analysis of environmental endotoxicosis-related SBP, DBP, and heart rate hemodynamic parameters, which were expressed by a higher level of PMW both in the group of patients treated with heparin, monopril and propranolol and PCI, as well as in PCI-treated subjects at the time of their hospital admission, showed no statistically significant difference ($p > 0.05$). The groups included those patients

Table 1

Characteristics of patients working in environmentally unfriendly conditions

Environmentally stressful Conditions (n=42)	Monopril + propranolol + heparin (n=21)		PCI (n=21)	
	Number of patients	Work experience (years)	Number of patients	Work experience (years)
1. Machine engineering	4	10.20±2.35	4	10.31±3.10
2. Oil and gas processing industry	6	10.40±6.30	5	10.20±2.20
3. Chemical plant	4	10.56±4.60	3	10.49±3.0
4. Motor vehicle repair workshops	5	10.40±2.30	6	10.23±2.10
5. "Electroterm" plant	2	10.05±1.40	3	10.50±2.10
Total	21	10.31±3.30	21	10.23±2.60
Admission period	1-5 h		1-5 h	

who suffered mainly of hyper- and eukinetic hemodynamic variants (tab. 2.)

A significantly low level of PMW was recorded in patients from the 1st group, over 12 hours, viz. from 0.58 ± 0.03 to 0.29 ± 0.01 units (p <0.001). A reduction in blood pressure was also registered, ranging between 138.0 ± 2.3 / 86.7 ± 1.3- to 123.0 ± 2.3 / 78.7 ± 1.7 mm Hg and which dropped up to 118.0 ± 1.8 / 75.9 ± 3.2 mm Hg until the end of the study (p> 0.001). Three patients from this group exhibited high level of PMW, though the blood pressure showed a decreasing tendency (up to 100/60 mm Hg). After the treatment, the level of PMW rapidly decreased (0.24 ± 0.11 unit), whereas blood pressure increased up to 115/65 mm Hg. PCI contributed to a slight decrease in PMW and blood pressure. The level of PMW decreased from 0.59 ± 0.6 to 0.56 ± 0.04 units, within 24 hours (p> 0.05), whereas blood pressure revealed 124.8 ± 3.4 / 74.00 ± 2.40 mm Hg, and 118.0 ± 1.7 / 75.1 ± 1.6 mm Hg on the 7th day (p <0.001). Changes of central hemodynamics and PMW during the treatment have been presented in table 2 (p> 0.05).

A significant decrease in heart rate among the 1st group patients was recorded over 24 hours, viz. from 100.0 ± 1.2 to 75.20 ± 1.40 beats/ minute, thus being of 74.10 ± 1.9 beats / minute at the end of the study. No heart rate decrease was registered in the 2nd group over 24 hours. However, it reduced up to 75.30 ± 2.1 beats/ minute within 72 hours.

The changes in the left ventricular (LV) function indices during the study are presented in table 2. The 1st group revealed a progressively decreasing EDV that was statistically significant difference in both the index before the treatment for MI (p <0.001), and the study results obtained in the 2nd group (p <0.01). Patients treated with just PCI also showed a decrease in EDV. 84% of patients from the 1st group and 75% of patients from the 2nd group showed a reduced LV EDV. 16% of patients of the 1st group and 25% of the 2nd group exhibited no decrease in both EDV index and its dynamics.

LV ESV in both the 1st group and the 2nd group decreased by 43.3% and 32.9% of patients, respectively, with a significant difference of p <0.01.

Indicators of the LV systolic function revealed no difference between groups (p <0.005). By the end of the 1st day and on the 7th day of the follow-up period, patients from the 1st group showed an increased EF (45.40 ± 2.5% and 61.91 ± 1.12%, respectively), which differed from the indicators of the 2nd group (45.90 ± 2.10% and 54.31 ± 1.11%). A decrease in the LV contractile function was recorded in 2 patients (9.5%) of the 1st group and in 10 patients (38.1%) of the 2nd group.

The values of LV LCDI were also different. Following a significant decrease in both the volume of myocardial deterioration and the severity degree of myocardial asynergy in patients from the 1st group, LV LCDI also exhibited lower values (1.88 ± 0.10- before treatment, and 0.81 ± 0.21 at the end of therapy). The indicators of LV LCDI from the 2nd group decreased from 1.78 ± 0.13 to 0.90 ± 0.12. (p> 0.001). Thus, a significant decrease in LV LCDI was registered in 97% of patients from the 1st group, and 57% of patients from the 2nd group .

Table 2

The dynamic patterns of central hemodynamic, LV systolic function and APM indices (M ± m)

Indices	Monopril + propranolol + heparin + PCI (n = 21)					PCI(n=21)					P_1 P_2
	Time elapsed since the start of treatment										
	Before treatment	12 h	24 h	72 h	7 day	Before treatment	12 h	24 h	72 h	7 day	
SBP mm Hg	138,0±2,3 P>0,05	123,0±2,3	126,0±1,6	125,2±1,6	118,0±1,8	140,4±2,2 P>0,05	122,2±2,1	124,8±3,4	125,5±2,2	118,0±1,7	<0,001 >0,05
DBP mm Hg	86,7±1,3 P>0,05	78,7±1,7	73,7±1,6	75,1±3,3	75,9±3,3	84,9±1,2 P>0,05	65,0±1,0	74,00±2,4	72,4±1,3	75,1±1,6	=0,05 >0,05
PMW, IU	0,58±0,03 p>0,05	0,29±0,01	0,30±0,06	0,28±0,02	0,26±0,05	0,59±0,06 P>005	0,56±0,04	0,54±0,03	0,49±0,03	0,46±0,02	<0,001 <0,01
HR beats/min	100,0±2,4 P>0, 05	88,6±2,80	75,20±1,40	76,72±2,1	74,10±1,90	98,6±3,10 P>0,05	96,50±2,2	96,19±2,00	75,32±2,10	75,30±2,10	<0,01 >0,05
ESV, ml	90,20±2,90 P>0,05	70,23±2,31	65,90±2,42	53,2±2,61	51,5±2,60	89,33±2,6 P> 0,05	78,5±2,31	75,20±2,42	68,20±2,1	65,10±2,4	<0,001 <0,01
EDV, ml	165,10±2,90 P>0,05	150,11±2,29	143,71±2,15	135,82±2,17	134,76±2,1	164,18±2,00 P>0,05	159,82±2,16	155,92±2,12	151,82±2,16	148,92±2,11	<0,001 <0,01
EF, %	45,40±2,5 P>0,05	53,20±0,10	54,8,±1,41	60,81±1,17	61,91±1,12	44,80±2,30 P>0,05	45,73±1,61	48,82±1,53	52,71±1,47	54,31±1,11	<0,001 <0,01
LCDI	1,88± 0,10 P>0,05	1,48± 0,23	1,35± 0,30	0,81± 0,17	0,81± 0,21	1,78± 0,13 P>0,05	1,73 ±0,17	1,52±0,15	0,87 ±0,12	0,90 ±0,13	< 0,01

Note: P – initial statistically significant difference; P_1- statistically significant difference between initial and final results; P_2 – statistically significant difference of the final study results between the two groups.

There were no significant differences in the clinical condition of patients on the 1st day. Acute heart failure was found in 2 patients from the 2nd group. One patient from the 1st group developed AHF on the 7th day. No restenosis, MI recurrence, and mortality cases were registered.

The group of patients, undergoing only PCI (the 2nd group) included 2 (9.5%) cases of recurrent MI; 4 patients developed restenosis, according to the repeated coronary angiography data; and 2 (9.5%) patients died. One patient died in the first 12 hours of PCI, the other one over 72 hours after the restenosis onset.

Therefore, the combined drug therapy and PCI used for revascularization of the coronary arteries contributed to reduction of PMW, ESV, EDV, LV LCDI, as well as an increase in LVEF, leading to a stabilization of hemodynamic patients without a critical decrease in their blood pressure during the MI follow-up. The combined therapeutic approach prevented the development of coronary artery restenosis, MI recurrence and mortality.

Discussion

Recently, the impact of environmental endotoxicosis has been highlighted regarding the hypoxia in peri-infarction area, myocardial metabolic impairment, and volume of myocardial deterioration, which leads to a change in LV systolic and diastolic function, as well as to the development of various complications, which are proportional to the degree of environmental endotoxicosis and volume of myocardial involvement [1, 5- 7].

Currently, in order to determine the degree of environmental endotoxicosis and the overall toxicity degree within the body, the PMW is being studied. The average peptide molecule is a marker of ischemic toxin, which impairs microcirculation, has a toxic effect on cardiomyocytes, and enhances the ischemic zone spread [1, 2]. Additionally, ecoendotoxicosis leads to an increase of PMW within the body, which inhibits the biosynthesis of proteins and the activity of a number of enzymes; oxidation and phosphorylation are impaired, the synthesis of adenine and glucose metabolism are also inhibited. The treatment failure of ecoendotoxicosis-related myocardial infarction is mainly due to insufficient assessment of the severity degree of endotoxemia associated with large doses of xenobiotic intake and anthropological-emergency factors, as well as the catabolic products derived from the peri-infarction area [1].

Therefore, cardiologists are searching for new drugs and methods to prevent complications and improve prognosis of AMI, which remains an urgent problem worldwide [7, 8, 10]. Recently, thrombolytic therapy and angioplasty (PCI) of the infarct-related coronary artery have been used [3, 9-12]. However, arrhythmia and reperfusion syndrome might occur in patients following a thrombolytic drug treatment. After drug reperfusion, remodeling of residual stenosis of the infarct-related coronary artery continues over the following week [9, 10]. There are evidences that a standard PCI, performed immediately after a successful thrombolysis, might increase the frequency of complications, namely of AHF, restenosis, and recurrence of MI.

The combined drug and PCI treatment does not only restore the coronary blood flow but also helps to restore the local kinetics of myocardium segments in the peri-infarction area [9, 10], as well as to reduce the level of PMW [1, 4, 6]. The clinical data and our study results have revealed that the use of β-adrenergic antagonists, particularly of propranolol [1] and metoprolol succinate [7, 8] prior to PCI and coronary bypass surgery are considered essential for myocardial protection and thus, reducing the mortality rate in this category of patients [12]. However, due to the high BP and negative inotropic actions, they were applied in only 20-35% of patients with MI (1, 7, 8), which is contrary to specialized literature data [7, 8] and our experience. Thus, if reasonable contraindications are followed, the use of propranolol or metoprolol succinate does not result in negative outcomes [1, 7]. Moreover, propranolol, which improves lymphocirculation, also helps in removing the eco- and endotoxins out from the peri-infarction area, and in reducing the systolic and diastolic LV functions.

In recent years, much attention has been paid to the use of ACE inhibitors in AMI, aimed at preventing the post infarct left ventricular remodeling and AHF [1, 2, 6]. Monopril (fosinopril) is the latest representative of ACE inhibitors, which is widely used in treatment of hypertension and congestive heart failure.

Thus, based on the research data, we concluded that the combined use of propranolol with monopril, heparin and PCI can reduce eco-endotoxicosis, restore myocardial blood flow, and improve LV systolic function. It also might increase the values of SI, CI, EF in patients, stabilize their SBP, DBP and heart rate. It also shows a more favorable impact on the clinical course of the disease.

Conclusions

1. The group of patients working under environmentally unfriendly conditions, and who underwent treatment with heparin, propranolol and monopril along with PCI at early stages of AMI, showed a rapid decrease in PMW, central hemodynamic stabilization, reduced systolic and diastolic LV functions, which might lead to early LV remodeling.

2. The combined use of heparin, propranolol and monopril together with PCI in the early stages of AMI patients, working in environmentally stressful conditions, revealed a more favorable clinical course of the disease; whereas MI recurrence, infarction-related coronary artery restenosis and mortality cases were not recorded.

3. The group of patients, subjected to PCI only, exhibited a high incidence of AHF, MI recurrence, infarction-related coronary artery restenosis, and mortality cases.

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Authors' ORCID iDs and academic degrees
Mamedgasan Agaev – https://orcid.org/0000-0002-7453-0843, MD, PhD.
Tarana Agaeva – https://orcid.org/0000-0003-0152-6074, MD, PhD.

Authors' contributions
MA conceptualized the project and designed the research. TA interpreted the data and drafted the manuscrit. All authors revised and approved the final version of the manuscript.

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Ethics approval and consent to participate
The study was approved by the ethics committee of Azerbaijan Medical University and *Abdulaev* Institute of Cardiology, Baku, Azerbaijan. The consent was received from every patient.

Conflict of Interests
No competing interests were disclosed.

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Automatic amperometric titration method for quantitative determination of zinc oxide in ointments

[1]Vasile Oprea, [2,3]Vladimir Valica, [1]Constantin Cheptanaru, *[2,4]Mihai Nistorica, [5]Serghei Oprea, [3,4]Liviu Movila

[1]Department of General Chemistry, [2]Department of Pharmaceutical Chemistry and Toxicology
[3]Scientific Center for Drug Research, *Nicolae Testemitsanu* State University of Medicine and Pharmacy
[4]Medicines and Medical Devices Agency, Chisinau, the Republic of Moldova
[5]Department of Informatics and Information Management, Academy of Economic Studies of Moldova Chisinau, the Republic of Moldova

Authors' ORCID iDs, academic degrees and contributions are available at the end of the article

*Corresponding author: nistoricamn@gmail.com
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Abstract

Background: Zinc is an important trace mineral in human body, therefore development of new methods for zinc determination in drugs is an actual task.
Material and methods: Assay of standard solutions of zinc ions and solutions of ointments to be analyzed was performed automatically using the titrator TITRION, which recorded, then displayed on the screen each amperometric titration curve. The titration curves had a sudden break in the current intensity at the equivalence point, caused by anodic oxidation of the titrant and cathodic reduction of H^+ at pH 3 – 4. The stoichiometry of Zn^{2+} ion sedimentation reaction with $[Fe(CN)_6]^{4-}$ ions was studied and confirmed in the presence of the background electrolyte and the ZnO calculation formulas in the analyzed ointments were deduced.
Results: Two different ointment masses were weighed for the Zoxitin ointment and the separate calculation results were 0.4172±0.0051 g/g and 0.4212±0.0051 g/g of ointment, and in the zinc oxide ointment ω(ZnO) was 10.208±0.078 %.These results were compared with the results of ZnO analysis in ointment by the classical complexometric titration method. For the Zoxitin ointment m(ZnO) was 0.4223±0.007 g/g of ointment, and for the ointment with ZnO ω(ZnO) was 10.18±0.11%.
Conclusions: An automatic amperometric titration method was developed to quantify ZnO in ointments. The method is based on the sedimentation reaction of zinc ions with potassium ferrocyanide solution in presence of acetate buffer solution as background electrolyte.
Key words: automatic amperometric titration, zinc oxide, quantitative determination of Zn.

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Introduction

Zinc is a vital element for a healthy immune system, having an important role for cell growth, cellular metabolism promoting healthy growth in childhood, increasing resistance to infections and wound healing. Medicines with zinc content are usually for internal or external use. Zinc can also be found in preparations for ophthalmic use or in solutions for parenteral use in total parenteral nutrition.

Topically used, zinc-containing preparations exhibit emollient, anti-inflammatory, antiseptic and protective action. It contributes to the reduction and elimination of local manifestations of exudation, inflammation and irritation.

In this context, the elaboration of the methods of quantitative analysis of zinc in order to ensure the quality of pharmaceutical forms remains current.

Material and methods

The Zoxitin ointment (manufacturer Farmaprim, the Republic of Moldova) containing ZnO as the active substance of 400 mg/g of ointment and the ZnO ointment prepared by the pharmacy of *Nicolae Testemitsanu* State University of Medicine and Pharmacy, the Republic of Moldova with the ZnO mass fraction of 10 %.

Laboratory vessels: volumetric flasks with different capacities, conical flasks and beakers with capacities of 50 and 100 ml, two DAPpette automatic pipettes with capacities of 100 – 1000 µl and 1000 – 5000 µl. The masses of the ointments to be analyzed, the masses of ZnO and $K_4[Fe(CN)_6]\cdot 3H_2O$ samples used for the preparation of the analyte and standard solutions were weighed into conical flasks or glass vials using the RADWAG AS 110.RI balance.

For the determination of ZnO in the nominated ointments, the automated amperometric titration kit named titrator TITRION (manufacturer ECONIS EXPERT, Russia) was used in the titration curve recording mode. This titrator is designed to perform automated volumetric assay of solutions, in which oxido-reduction processes take place, using amperometric method based on the measurement of the current intensity in the circuit of a system of two Pt

electrodes to the application of an external voltage. The general aspect of this automated amperometric titration kit is shown in fig. 1.

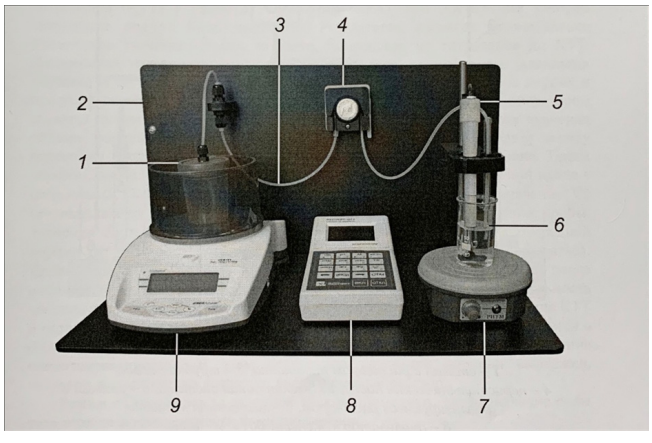

Fig. 1. External appearance of the kit for automatic amperometric titration – titrator TITRION.

1 – beaker with the titrant solution, 2 – the case of titrator TITRION, 3 – pump tube, 4 – peristaltic pump, 5 – electrode system, 6 – beaker with solution to be analyzed, 7 – magnetic stirrer, 8 – liquid analyzer EXPERT-001, 9 – balance.

The "brain" of this titrator is the liquid analyzer EXPERT-001, which contains programs for assay, measuring the current intensity (μA) and the solution fractions (ml) of the titrant added to the analyte solution over certain time intervals (s), announces the end of the titration, displays the value of the current intensity and the total volume of the titrant consumed for quantitative determination, records and displays on the screen the titration curve, which is processed by the operator to determine the titrant equivalence volume [1].

Preparation of solutions

All the solutions used in the study were prepared from the reagents, classified as "chemically pure", but the potassium ferrocyanide was recrystallized from the saturated aqueous solution upon addition of ethanol [2].

Standard and auxiliary solutions

ZnO standard solutions with molar concentrations equal to 0.005 mol/l and 0.01 mol/l were prepared in the study as follows. ZnO masses equal to 0.1017 g and 0.1627 g, respectively, were quantitatively passed into 250 ml and 200 ml volumetric flasks, and 4.0 ml of solution with c(HCl)=2 mol/l were added. The ZnO masses were dissolved, the solutions were diluted with distilled water up to the mark and homogenized. The solution with c(HCl)=2 mol/l was prepared from fixanal (c(HCl)=0,1 mol/dm^3).

In the automatic amperometric titration method as the titrant in the studying of the stoichiometry of the Zn^{2+} ion sedimentation reaction as well as to determine ZnO in the analyzed ointments, potassium ferrocyanide solutions with molar concentrations equal to 0.01 mol/l and 0.04 mol/l were used. These solutions were prepared by calculating, accurately weighing the masses of these samples of complex salt of $K_4[Fe(CN)_6]\cdot 3H_2O$, which had been quantitatively passed into a 50 ml volumetric flask, dissolved, diluted with distilled water to the mark and homogenized. The titrant solutions were used on the day of their preparation.

Zinc oxide in the solutions for the analysis of the ointments was quantitatively determined by the complexometric titration method. In this method, the disodium salt of EDTA, which is a crystalohydrate, used in general formula $Na_2H_2Y\cdot 2H_2O$ [3-5], is used as the primary standard and is also called disodium edetate [5]. Standard solutions with c(1/2 Na_2H_2Y)=0.05 mol/l and c(1/2 Na_2H_2Y)=0.1 mol/l were used in the analysis. The first solution was prepared by accurately weighing the calculated sample of $Na_2H_2Y\cdot 2H_2O$ crystalohydrate with a mass of 0.9306 g, which was quantitatively passed into a 100 ml volumetric flask, dissolved, diluted to the mark with distilled water and homogenized. The second solution was prepared from fixanal (c(1/2 $Na_2H_2Y\cdot 2H_2O$)=0.1 mol/l).

Auxiliary solutions were also used in the study. All quantitative determinations in the automatic amperometric method were performed in the presence of acetate buffer with different pH values, which was used as background electrolyte. These solutions were prepared by mixing the sodium acetate solution containing a constant mass of $Na(CH_3COO)\cdot 3H_2O$, with different volumes of concentrated CH_3COOH acid solution.

In the complexometric titrations to maintain pH=10, the ammonia buffer solution was used. This solution was prepared as follows. Ammonium chloride with mass of 5.4 g was dissolved in a minimum volume of distilled water, 35 ml concentrated NH_3 solution was added thereto and diluted with distilled water to 100 ml of buffer solution. The volume of the titrant in this method was measured using a 2.00 ml microburette.

The pH of the analyzed solutions was measured using the I-160M ionometer, connected with a glass indicator electrode and a silver–silver chloride reference electrode.

Preparation of Zoxitin ointment solution to be analyzed

In a 100 ml conical flask, about 0.2 – 0.3 g (exact mass of ointment), 4.0 ml of solution with c(HCl)=2 mol/l and 25 ml distilled water were added. The conical flask was installed on a hot asbestos plate, and the solution was heated to boiling temperature, kept near this temperature, and shaken until the entire ointment base together with ZnO dissolved. The solution was cooled then under the tap, filtered, the filtrate was taken up in a 200 ml volumetric flask. The conical flask and the filter were washed 2 – 3 times with 15 – 20 ml of distilled water, then the solution in the volumetric flask was diluted with distilled water to the mark and homogenized.

The solution to be analyzed of ZnO ointment prepared in the pharmacy of the *Nicolae Testemitsanu* State University of Medicine and Pharmacy was prepared analogously to the Zoxitin ointment solution except that about 1 g (exact mass) of the ointment was taken for the assay.

Results and discussions

The previous publications [6-10] have been devoted to the elaboration, completion and masking of interfering ions in the quantitative determination of Zn in different electrolytes by the amperometric titration method with $K_4[Fe(CN)_6]$ solution. For this, anodic oxidation of titrant excess and background electrolytes with different composition and concentrations were used, as well as the composition of the sediment, formed during assay, was all different [6].

The information about an amperometric titrator developed by ECONIS EXPERT for didactic purposes was published in the indication [11]. The titrator is equipped with two Pt indicator electrodes. Zn assay was performed with $K_4[Fe(CN)_6]$ solution in the presence of acetate buffer solution (pH 3 – 4) as background electrolyte and the sediment composition was complex salt $Zn_2[Fe(CN)_6]$.

Method of preparation and titration of the solutions to be analyzed using the titrator TITRION

Using an automated pipette, a certain volume of standard solution of Zn^{2+} ions or solution to be analyzed of the ointment was measured and added to a 50 ml titration beaker. In beaker, 10.0 ml of distilled acetate solution and distilled water were added with a pipette, so that the final volume of the solution taken for titration was 20.0 ml. The solution was then automatically titrated with standard $K_4[Fe(CN)_6]$ solution using the titrator as indicated in the operating compendium [1].

All amperometric determinations with standard $K_4[Fe(CN)_6]$ solution were performed in the titration curve recording mode and the voltage applied to the indicator electrodes was 0.2 V. The curves, which were obtained as a result of assay, had a very pronounced turning of the current intensity at the equivalence point. They were processed using the liquid analyzer EXPERT to determine the equivalence volume of the titrant [1]. As an example in fig. 2 are shown the appearance of an amperometric titration curve of a solution to be analyzed with $K_4[Fe(CN)_6]$ solution and the determination of equivalence volume of titrant.

However, at amperometric dosage with this titrant solutions, where the concentration of Zn^{2+} ions was in the order

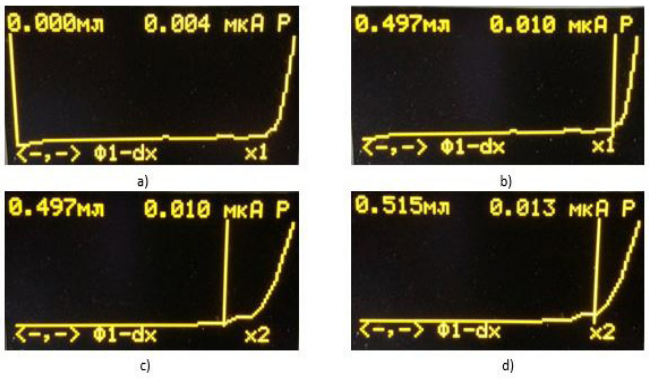

Fig. 2. Appearance of the titration curve of a solution to be analyzed with ZnO (a), its processing to determine the titrant volume in the near of the equivalence point (b and c) and the equivalence point (d).

Fig. 3. The appearance of a Zn^{2+} ion solution titration curve with $K_4[Fe(CN)_6]$ standard solution ($c(Zn^{2+}) = 5 \cdot 10^{-3}$ mol/l; $c(K_4[Fe(CN)_6]) = 0.04$ mol/l; pH 3.5).

of $n \cdot 10^{-3}$ mol/l, the appearance of the titration curves was similar to that shown in fig. 3. This aspect of the titration curve up to the equivalence point of the titrant can probably be elucidated on the basis of the equilibrium that results in the solution at the formation of the sediment [6, 12].

In studying the stoichiometry of the Zn^{2+} ion sedimentation reaction with the $K_4[Fe(CN)_6]$ standard solution by the amperometric titration method, the preparation of the solution for titration and its assay was carried out according to the procedure outlined above. The data obtained are shown in tab. 1.

Table 1

Study of stoichiometry of Zn^{2+} ion sedimentation reaction with $K_4[Fe(CN)_6]$ standard solution by the amperometric titration method with two Pt indicator electrodes

№	V (Zn^{2+}) ml	n (Zn^{2+})·10^2 mmol	V ($K_4[Fe(CN)_6]$) ml	n ($K_4[Fe(CN)_6]$)·10^2 mmol	$\frac{n(Zn^{2+})}{n(K_4[Fe(CN)_6])}$
1	0.50	0.25	0.125	0.125	2.000
2	1.00	0.50	0.252	0.252	1.984
3	1.50	0.75	0.372	0.372	2.016
4	2.00	1.00	0.498	0.498	2.008
5	2.50	1.25	0.619	0.619	2.019
6	3.00	1.50	0.748	0.748	2.005
7	4.00	2.00	1.004	1.004	1.992
8	5.00	2.50	1.243	1.243	2.011

($c(Zn^{2+})$=0.005 mol/l; $c(K_4[Fe(CN)_6)$=0.01 mol/l; $\Delta\varphi$=0.2 V; pH = 3.7)

Table 2

Study of the influence of pH of the solution on the stoichiometry of Zn^{2+} ion sedimentation reaction with $K_4[Fe(CN)_6]$ standard solution by the amperometric titration method with two Pt indicator electrodes

№	pH	V (Zn^{2+}),ml	n (Zn^{2+})·10^2 mmol	V ($K_4[Fe(CN)_6]$) ml	n ($K_4[Fe(CN)_6]$)·10^2 mmol	$\frac{n(Zn^{2+})}{n(K_4[Fe(CN)_6])}$
1	3.17	2.00	1.00	0.497	0.125	2.012
2	3.33	2.00	1.00	0.502	0.252	1.992
3	3.55	2.00	1.00	0.498	0.372	2.008
4	3.87	2.00	1.00	0.499	0.498	2.004
5	4.02	2.00	1.00	0.502	0.619	1.992
6	4.21	2.00	1.00	0.512	0.748	1.953

($c(Zn^{2+})$=0.005 mol/l; $c(K_4[Fe(CN)_6]$=0.01 mol/l; Δφ =0.2 V)

It is confirmed that in the presence of the acetate buffer solution as a background electrolyte the ratio $n(Zn^{2+})$: $n(K_4[Fe(CN)_6])$ = 2 : 1 and the sedimentation reaction takes place in the solution after the ionic equation: $2\ Zn^{2+} + [Fe(CN)_6]^{4-} = Zn_2[Fe(CN)_6]$.

It does not influence this ratio nor the pH of the solution in the range of values 3.17 – 4.02 (tab. 2), which was investigated in analogy with the stoichiometry of the Zn^{2+} ion sedimentation reaction with $K_4[Fe(CN)_6]$.

The mass of zinc oxide (m(ZnO), g/g) in one gram of Zoxitin ointment, based on the stoichiometry of the sedimentation reaction, can be calculated according to the formula:

$$m(ZnO) = 2 \times c(K_4[Fe(CN)_6] \times V(K_4[Fe(CN)_6] \times M(ZnO) \times \frac{V_0 \times 10^{-3}}{V_1 \times m_u}, \quad (1)$$

in which: $c(K_4[Fe(CN)_6])$ – the molar concentration of the titrant solution, mol/l;

$V(K_4[Fe(CN)_6])$ – the titrant's volume of equivalence, ml;

M(ZnO) – molar mass of zinc oxide, g/mol;

V_0 – the capacity of the volumetric flask with Zoxitin ointment solution, ml;

V_1 – the analyte fraction of this ointment, taken for analysis, ml;

m_u – ointment mass, taken for analysis, g.

However, the equation of the sedimentation reaction of Zn^{2+} ions with $K_4[Fe(CN)_6]$ standard solution, shows that the equivalence factor of the titrant is equal to ½. In this case, the product $2 \times c(K_4[Fe(CN)_6]$ is equal to $c(\frac{1}{2}K_4[Fe(CN)_6]$ and equation (1) simplifies and turns into the relation:

$$m(ZnO) = T_{K_4[Fe(CN)_6]/ZnO} \times V(K_4[Fe(CN)_6] \times \frac{V_0}{V_1 \times m_u}, \quad (2)$$

in which: $T_{K_4[Fe(CN)_6]/ZnO}$ – the theoretical titre of the - potassium ferrocyanide solution relative to ZnO, g/ml. The other notes are shown above.

Table 3

Determination of the mass of zinc oxide in one gram of Zoxitin ointment by automatic amperometric titration method with two Pt indicator electrodes

a – m_u=0.2871 g

№	V_1, ml	V ($K_4[Fe(CN)_6]$), ml	m (ZnO), g/g
1	0.50	0.179	0.4057
2	0.75	0.181	0.4103
3	1.00	0.277	0.4186
4	1.25	0.370	0.4193
5	1.50	0.461	0.4180
6	2.00	0.562	0.4246
7	2.50	0.741	0.4199
8	3.00	1.113	0.4204

($T_{K_4[Fe(CN)_6]/ZnO}$ = 0.0016274 g/ml; V_0=200 ml; Δφ =0.2 V; pH 3.5)

b – m_u=0.3556 g

№	V_1, ml	V ($K_4[Fe(CN)_6]$) ml	m (ZnO), g/g
1	1.00	0.116	0.4247
2	1.50	0.175	0.4272
3	2.00	0.235	0.4302
4	2.50	0.276	0.4042
5	3.00	0.348	0.4247
6	3.50	0.401	0.4195
7	4.00	0.461	0.4220
8	4.50	0.518	0.4215
9	5.00	0.568	0.4159
10	6.00	0.692	0.4223

($T_{K_4[Fe(CN)_6]/ZnO}$ = 0.0065096 g/ml; V_0=200 ml; Δφ =0.2 V; pH 3.5)

Equation (2) was used to calculate the results of ZnO analysis in Zoxitin ointment analytical solutions by the automatic amperometric titration method with two Pt-

indicator electrodes. The results are presented in tab. 3a and tab. 3b. For quantitative determination of solutions to be analyzed of this ointment, as a titrant, standard solution of $K_4[Fe(CN)_6]$ with molar concentration of the equivalent equal to 0.02 mol/l and 0.08mol/l were used, by means of which the theoretical titre $T_{K_4[Fe(CN)_6]/ZnO}$ from equation (2) was calculated.

Results of ZnO analysis in the solutions to be analyzed for the two Zoxitin ointment masses in tab. 3a and tab. 3b have been processed statistically separately and the mean mass of ZnO in one gram of ointment consisted of (0.4172±0.0051) g/g (tab. 3a) and (0.4212±0.0051) g/g (tab. 3b), with a confidence interval of 95 %.

Using the mentioned titrator, the assay of the ZnO ointment solution, prepared in the pharmacy of Nicolae Testemitsanu State University of Medicine and Pharmacy, was also made. The results obtained are presented in tab. 4.

Table 4

Determination of zinc oxide mass in ZnO ointment solution, prepared in the pharmacy of *Nicolae Testemitsanu* State University of Medicine and Pharmacy, by automatic amperometric titration method with two Pt indicator electrodes

№	V_1, ml	V ($K_4[Fe(CN)_6]$), ml	ω (ZnO), %
1	0.50	0.173	10.247
2	1.00	0.349	10.336
3	1.25	0.435	10.306
4	1.50	0.515	10.168
5	2.00	0.696	10.306
6	2.50	0.862	10.212
7	3.00	1.024	10.109
8	4.00	1.371	10.151
9	5.00	1.694	10.034

($T_{K_4[Fe(CN)_6]/ZnO}$ = 0.0016274 g/ml; V_0=200 ml; m_u=1.099 g; Δφ =0.2 V; pH 3.5)

The mass fraction of ZnO (ω(ZnO), %) in this ointment was calculated according to the equation:

$$\omega(ZnO) = T_{K_4[Fe(CN)_6]/ZnO} \times V(K_4[Fe(CN)_6] \times \frac{V_0 \times 100}{V_1 \times m_u}, \quad (3)$$

The results of the analysis ω(ZnO) in tab. 4 were statistically processed and for this ointment the mean mass fraction of ZnO was (10.208±0.078) % for the 95 % confidence interval.

To evaluate and confirm the results obtained by the automatic amperometric titration method, the analytical solutions of these two ointments were also analyzed by the complexometric method. In a 100 ml titration flask a certain volume of the ointment solution was added, measured by means of an automatic pipette, 5 – 6 ml of ammonia buffer solution with pH=10, 20 ml distilled water, 0.05 g mixture eriochrome black T indicator and the obtained solution was titrated with standard solution of $Na_2H_2Y{\cdot}2H_2O$ with c($1/2Na_2H_2Y$) equal to 0.05 mol/l or 0.1 mol/l until the change from red to violet in blue [5]. The results obtained are presented in tab. 5 and tab. 6.

Table 5

Determination of the mass of zinc oxide in one gram of Zoxitin ointment by the complexometric titration method

№	V_1, ml	V (Na_2H_2Y), ml	m (ZnO), g/g
1	3.00	0.37	0.4194
2	3.50	0.43	0.4178
3	4.00	0.50	0.4251
4	4.00	0.51	0.4336
5	4.50	0.56	0.4232
6	5.00	0.61	0.4149

($T_{Na_2H_2Y/ZnO}$ = 0.004069 g/ml; V_0=200 ml; m_u=0.2393 g; pH 10)

Table 6

Determination of zinc oxide mass fraction in the ZnO ointment, prepared in *Nicolae Testemitsanu* State University of Medicine and Pharmacy, by the complexometric titration method

№	V_1, ml	V (Na_2H_2Y), ml	ω (ZnO), %
1	2.00	0.55	10.18
2	2.50	0.70	10.36
3	2.75	0.74	9.96
4	3.00	0.83	10.24
5	3.50	0.98	10.36
6	4.00	1.09	10.09
7	4.50	1.23	10.12
8	5.00	1.37	10.14

($T_{Na_2H_2Y/ZnO}$ = 0.002034 g/ml; V_0=200 ml; m_u=1.099 g; pH 10)

Calculations of the results of the complexometric analysis of ZnO mass in one gram of ointment (m(ZnO), g/g) Zoxitin was performed according to the relationship:

$$m(ZnO) = T_{Na_2H_2Y/ZnO} \times V(Na_2H_2Y) \times \frac{V_0}{V_1 \times m_u}, \quad (4)$$

in which: $T_{Na_2H_2Y/ZnO}$ – the theoretical titre of the titrant solution relative to ZnO, g/ml;

$V(Na_2H_2Y)$ – the titrant's volume of equivalence, ml.

The other marks are shown above.

All the notes in equation (4) remained the same for equation (5), after which the mass fraction of ZnO (ω (ZnO),%) was calculated in the solution of ZnO ointment, prepared in the SUMP «Nicolae Testemitsanu» pharmacy, using the complexometric titration method:

$$\omega(ZnO) = T_{Na_2H_2Y/ZnO} \times V(Na_2H_2Y) \times \frac{V_0 \times 100}{V_1 \times m_u}, \quad (5)$$

The results in tab. 5 and tab. 6, obtained by the complexometric titration method, were statistically processed. For the Zoxitin ointment the mean ZnO mass was (0.4223±0.007) g/g of ointment, and for the ZnO ointment prepared in the pharmacy of *Nicolae Testemitsanu* State University of Medicine and Pharmacy, the mean mass fraction of ZnO constituted (10.18±0.11) %. In both calculations, the confidence interval was 95%.

These results compared to the automatic amperometric titration method with two Pt-indicator electrodes are satisfactory. However, for one and the same 95% confidence level, the uncertainty of measurement of both m(ZnO) and ω (ZnO) from their mean values in ointment (see above) is lower in the automatic amperometric titration method compared to the classical complexometric titration method. This can be explained by the fact that in the electrochemical automated assay method of solutions of ZnO in ointments by means of the mentioned titrator, the equivalence volume of the titrant was determined more accurately than visually in the complexometric titration method.

Conclusions

1. A new method of automatic amperometric dosage of ZnO in ointments was developed using the titrator TITRYRON. The developed method can be recommended for the quantitative analysis of ZnO in ointments.

2. Quantitative analysis of ZnO in ointments was performed by two methods: automatic amperometric dosing with two Pt indicator electrodes by titrator TITRION and classical complexometric. Both methods yielded satisfactory results, but the accuracy limits from the mean value of ZnO in ointments were lower in the developed method compared to the complexometric titration method.

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Authors' ORCID iDs and academic degrees
Vasile Oprea – https://orcid.org/0000-0001-9273-7613, Chem PhD.
Vladimir Valica – https://orcid.org/0000-0002-1068-5504, MD, Pharm PhD.
Constantin Cheptanaru – https://orcid.org/0000-0003-3281-6613, Chem PhD.
Mihai Nistorica – https://orcid.org/0000-0001-9433-6055, Pharm D.
Serghei Oprea – https://orcid.org/0000-0002-5620-5131, Physics and Math PhD.
Liviu Movila – https://orcid.org/0000-0001-8540-9400, Pharm D.

Authors' contributions
VO designed the trial and interpreted the data. VV acquired and interpreted the data. CC interpreted the data. MN conducted/performed the laboratory work, drafted the first manuscript. SO described and processed the physics data. LM conducted/performed the laboratory work, revised the manuscript critically. All the authors revised and approved the final version of the manuscript.

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No competing interests were disclosed.

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Estimating the clinical needs for tissues and cells in the Republic of Moldova

Tatiana Timbalari

Transplantology Department, Transplant Agency, Laboratory of Tissue Engineering and Cells Cultures
Nicolae Testemitsanu State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova

Authors' ORCID iDs, academic degree and contribution are available at the end of the article

Corresponding author: tatiana.timbalari@gmail.com
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Abstract

Background: Safe and sustainable supply of tissues and cells for human application is an essential pillar in modern medical assistance and a priority for the health authorities both at national and international level.
Material and methods: The study protocol for the needs assessment in tissue and cell grafts for the healthcare system was approved by the Ethic Committee. The survey of 161 surgeons was carried out in 24 institutions, through specially developed questionnaires. In order to validate some data obtained through questionnaires, the analysis of already existing national data and sources was performed.
Results: Most surgeons (93.4%) declare that the institution they work at has been provided with a sufficient number of grafts for all patients needing transplantation. Despite this, the estimation of surgeons' opinion regarding the need for grafts showed that 59.4% of respondents consider that they have enough grafts, 21.7% of respondents deal with exceptional cases of lack of grafts, 13.2% respondents consider that there are sufficient grafts for urgent patients and, sometimes, for non-urgent patients.
Conclusions: The study revealed that increasing the number of tissue transplantations will contribute to improve the patients' life quality and increasing the number of transplanted patients over the next 5 years is a priority measure in the management of the institutions included in the survey.
Key words: tissue and cell grafts, clinical needs, transplantation, transplant services.

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Introduction

Transplantology as a medical science and transplantation per se have recorded a significant progress over the past decades. In analysts' opinion, transplantation is an indicator of the level of development of medical industry, the scientific and practical potential of a state and the level of maturity of society [1]. As to organs, the demand for certain types of tissues and cells to be transplanted goes beyond the available supply.

World Health Organization (WHO) presented two reports – First and Second Global Consultation on Regulatory Requirements for Human Cells and Tissues for Transplantation (2004 and 2006, respectively) providing an overview regarding the relevance and development of this field in different countries. The First WHO Report defines the tissue and cell grafts as a specific class of medical products with an important therapeutic value where almost no substitutes for restoring vital functions exist [2]. Many countries face a deficit of standards and regulations relevant for this field and they also face a lack of necessary grafts, for instance corneal grafts. The other Report highlights the importance of systems safe for traceability and biovigilance, underlining the fact that, for instance, annually, in Spain almost 10.000 patients get bone, tendon or corneal grafts [3].

The American Association of Tissue Banks (AATB), which includes more than 120 accredited tissue banks, says that the tissue banks have been established to satisfy critical medical needs for tissue grafts in order to save or to improve life of more than a million Americans yearly [4].

AATB's Activity Report for 2017 shows that over the last 10 years there has been noted a general increase in donors and grafts procurement. The total number of actual tissue donors (both alive and deceased ones) has rapidly increased by 92%: from 30.380 in 2007 up to 58.339 in 2015. The total number of grafts distributed has also gone up from 2.496.010 in 2007 up to 3.294.066 in 2015, representing 32% [4, 5]. The musculoskeletal grafts remain to be the most requested ones and represent 71% of the total number of tissues distributed in 2015. In the period 2012-2015, the number of grafts distributed registered a rise in those 5 categories out of six in whole, except for cardiac tissues: musculoskeletal, skin, soft tissues, vessel, tissues from alive donors have increased up to 122%.

Spain has the highest number of deceased donors. If in the middle of the '90's, it counted 27 donors per million populations (pmp), in 2017 this rate went up to 46.9 pmp [6, 7]. Tissue donation has increased in the period 2011 – 2016: cornea – from 60 pmp up to 82.4 pmp, musculoskeletal tissue – from 44.8 pmp up to 57 pmp, skin tissue – from 4.5 pmp up to 7.3 pmp [8, 9].

Croatia has successfully introduced different elements of the best European practices (Spanish model, Eurotransplant system, etc.) and recorded a sustainable increase in the rate of pmp donors, registering in 2017 a rate of 33 donors pmp [10]. In 2011 – 2016, the corneal tissue donors have increased significantly from 2.7 pmp up to 69.9 pmp [8, 9].

In other European Union countries (EU countries), the number of donors and tissues transplants have also increased. For instance, in France, in the period 2011-2016, the tissue donation has significantly increased, namely the musculoskeletal tissue from 1.3 pmp up to 402 pmp, the number of grafts distributed for transplantation has risen from 27997 up to 47528, representing 41% [8, 9].

The increase in possibility to use human material in various forms for the others' benefit during the medical treatment has put significant pressure on the EU countries to satisfy this demand. The demand of human material is obviously variable as scientific developments make available more forms of treatment, it is likely that demand for such treatment to go up, meanwhile the development of alternate forms of treatment could result in decrease of demand. People's expectations as to what the medical sciences could reach put additional pressure on that supply [11].

Safe and sustainable supply of tissues and cells is an essential pillar in modern medical assistance in the EU countries and a priority for the health authorities both at national and the EU level [12-14]. In more situations, a tissue or cell transplant is the best or the single therapeutic option for patients. The supply and offer of human tissues and cells is a task of management during which medical, financial and social aspects should be constantly balanced as for the organization to continue to work and to be accepted by society [15].

The history in different types of tissue and cell banks underscore the complicated and interconnected ways the tissues and cells donated by one person may be used to help others or themselves [16, 17].

The main aim to preserve human material for transplantation in a bank is to satisfy the clinical demand of tissues and cells [18, 19]. The national health authorities are responsible to ensure that the patients' needs are satisfied with a safe, qualitative and appropriate source of tissues and cells [20].

The goal of this study was to assess the needs for tissue and cell grafts for the healthcare system in order to ensure the functioning of the transplant services provided to the population.

Material and methods

The study was performed to evaluate the opinions of the doctors of surgical profile, whether or not involved in the transplant services, regarding the needs for tissue and cell grafts for the healthcare system, through specially developed questionnaires, consisting of 35 questions. The volume of the representative sample of the doctors of surgical profile was calculated based on the classical formula, proposed for the random non-repetitive survey with an admitted error of 5% and a non-response rate of 10.0%. In order to validate some data obtained through questionnaires, we used the case studies and the analysis of already existing national data and sources. The study protocol was approved by the Ethic Committee of the Nicolae Testemitsanu State University of Medicine and Pharmacy, Chisinau (No. 2, 27.10.2016).

The survey of 161 doctors of surgical profile aged 25 – 81 was carried out in 24 medical-sanitary institutions, including 20 public institutions (9 republican, 2 municipal and 9 district) and 4 private institutions. In order to carry out the tissue and cell donation and transplantation activities, 10 from 24 medical-sanitary institutions are authorized by the Ministry of Health: 8 public and 2 private institutions. Their annual activity reports submitted to the Transplant Agency were used for the analysis.

In the group of doctors of surgical profile were included: 24 (14.9%) transplant coordinators and persons in charge of transplantation from medical-sanitary institutions with activities in the field of tissues and cells donation and transplantation, 52 (32.3%) orthopaedic traumatologists, 27 (16.8%) general surgeons, 15 (9.3%) ophthalmologists, 7 (4.3%) combustiologists, 36 (22.4%) doctors with another surgical profile – oncologists, neurosurgeons, gynecologists, urologists.

Of the total number of surgeons, 125 (77.6%) were men and 36 (22.4%) were women. Depending on age and gender, the respondents in the study group were distributed as follows: 25 (15.5%) persons were aged within 25 – 34 years (15 – 60.0% men and 10 – 40.0% women), 93 (57.8%) persons – within 35 – 54 years (73 – 78.5% men and 20 – 21.5% women), 43 (26.7%) persons – within 55 – 81 years (37 – 86.0% men and 6 – 14.0% women). The average age of surgeons was 46.84 ± 0.9 years. The average age was statistically significantly higher in men – 47.78 ± 0.9 years, compared with women – 43.58 ± 2.1 years (from 25 to 70 years; $p < 0.05$).

Statistical processing of the material was based on the special files elaborated where the primary data were coded – the results of the questionnaires, the data from the primary documents of the medical-sanitary institutions and the data from the databases of the Transplant Agency. The primary materials of the study were computerized using the software "Statistical Package for the Social Science" version 20.0 for Windows (SPSS, Inc., Chicago, IL, 2011) by methods of variation, correlation and discriminant analysis. Differences with the bilateral p-value <0.05 were considered statistically significant.

Results and discussion

To assess the needs for tissue and cell grafts, 139 (86.3%) surgeons from the public medical-sanitary institutions and 22 (13.7%) surgeons from the private medical-sanitary institutions have been interviewed. Out of the mentioned surgeons, 106 (65.8%) respondents were from the medical–sanitary institutions with activities in the field of tissues and

cells donation and transplantation, and 55 (34.2%) respondents were from the medical-sanitary institutions without activities in this field.

The survey showed that to carry out donation and transplantation activities does not depend on the type of financing, legal form of medical institution: 13 (59.1%) respondents confirmed that transplant services are provided in private institutions and 93 (66.9%) respondents – in public institutions (p>0,05).

The estimate of frequency of different transplant types made was taken from the annual activity reports by the medical-sanitary institutions and based on the opinion of surgeons from the medical-sanitary institutions included in the survey.

Most surgeons – 99 (93.4%) – say that the institution they work at has been provided with a sufficient number of tissue grafts for all patients needing transplantation. Only 7 (6.6%) respondents consider that the provided tissue grafts are insufficient. Respondents from the public medical-sanitary institutions say that they are provided with sufficient tissue grafts to cover all patients needing transplant, statistically more frequently compared to respondents from private institutions: 91 (97.8%) and 8 (61.5%), respectively; p <0.001).

Despite this, the estimation of surgeons' opinion regarding the need for grafts showed the following: 63 (59.4%) respondents consider that their institutions have enough grafts, 23 (21.7%) respondents consider that their institutions deal with exceptional cases of lack of grafts, 14 (13.2%) respondents consider that there are sufficient grafts for urgent patients and, sometimes, for non-urgent patients, and 6 (5.7%) respondents consider that grafts are enough only for patients in emergency need (fig. 1).

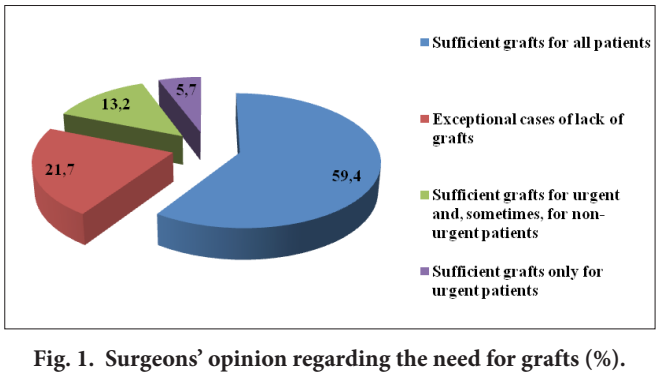

Fig. 1. Surgeons' opinion regarding the need for grafts (%).

The need to develop the tissue transplantation, especially the cornea transplant, results from the analysis of further increase in the cornea transplant waiting list that is presently performed in 5 medical-sanitary institutions with activities in the field of tissues and cells donation and transplantation [21]. The total annual number of cornea transplantations in average is 7.2±3.34 (from 1 up to 19 corneal grafting surgeries) (fig. 2).

Over ½ (24 – 51.1%) of surgeons would perform more corneal grafting surgeries if such grafts were provided by

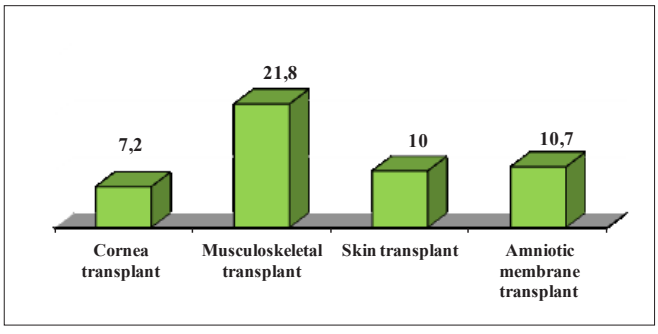

Fig. 2. Annual number of tissue and cell transplants performed (average value).

the tissue bank – in average 12.33±1.86 (from 4 up to 15 corneal grafts). In the private medical-sanitary institutions, this parameter is statistically more frequently encounted (6 – 100.0%) compared to the municipal medical-sanitary institutions (1 – 16.7%; p<0.001) and the republican medical-sanitary institutions (17 – 48.6%; p<0.001).

One of the reasons why the donation and transplantation activities are not performed is that there is no qualified medical personnel and appropriate medical equipment. The main reasons why the corneal grafting is not performed in the public and private medical-sanitary institutions is the lack of qualified medical staff and adequate medical equipment, problem that was highlighted by 28 (24.6%) surgeons, the lack of qualified medical staff – by 16 (14.0%) surgeons, the lack of adequate medical equipment – by 16 (14.0%) surgeons and other reasons (the institution is focused on other type of transplantation of human tissues or is not authorized to perform such surgeries) were mentioned by 54 (47.4%) surgeons (fig. 3).

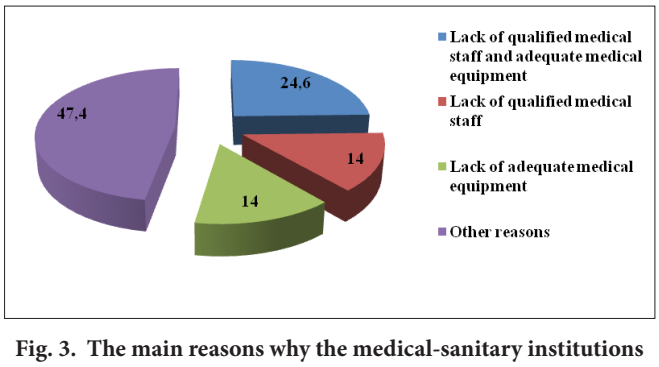

Fig. 3. The main reasons why the medical-sanitary institutions do not perform cornea transplantation (%).

According to the medical-sanitary institutions' authorization provided to perform activities in the field of tissues and cells donation and transplantation, the following results were obtained. Respondents from institutions with activities in this field mentioned that the corneal grafting is not performed due to the fact that there is no qualified medical personnel and no adequate medical equipment in 2 (3.4%) cases, no qualified medical staff – 10 (16.9%) cases, no adequate medical equipment – 6 (10,2%) cases and the institution has another transplant profile – 41 (69.5%) cases. Re-

spondents from institutions not performing activities in the field of tissues and cells donation and transplantation noted that the cornea transplantation is not performed due to the absence of qualified medical personnel and adequate medical equipment – 26 (47.3%) cases, qualified medical staff – 6 (10.9%) cases, adequate medical equipment – 10 (18.2%) cases and authorization for performing donation and transplantation –13 (23.6%) cases (fig. 4).

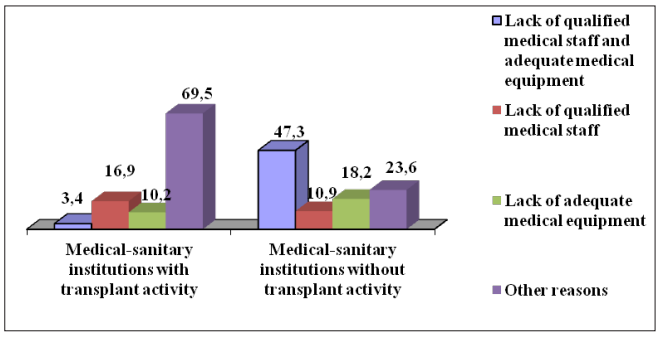

Fig. 4. The main reasons why the medical-sanitary institutions do not perform cornea transplantation (%) depending on the deployment activities in the field of tissue and cell donation and transplantation.

The musculoskeletal tissue transplantation is performed in 6 (60.0%) medical-sanitary institutions with activities in the field of tissues and cells donation and transplantation. The total annual number of musculoskeletal tissue grafting surgeries is in average of 21.83±6.48 (from 1 up to 43 musculoskeletal tissue grafting surgeries) (fig. 2). The cancellous bone or cortical bone transplantation was mentioned by 25 (32.9%) surgeons, where the bone and tendon transplantation was mentioned by 51 (67.1%) surgeons. Over 2/5 (34 – 44.7%) of surgeons would perform more musculoskeletal tissue grafting surgeries if those grafts were offered by the tissue bank. As to the republican medical-sanitary institutions this parameter is statistically more frequent (32 – 50.0%) compared to the private institutions (0 – 0%; p<0.001).

Moreover, over 4/5 (67 – 88.2%) of surgeons will need the provided bone grafts in other forms than the frozen ones (lyophilized, demineralized, morsel): statistically less often in the republican medical-sanitary institutions (55 – 85.9%) against private ones (7 – 100.0%; p<0.01) and municipal ones (5 – 100.0%; p<0.01).

Wang W, Yeung KW. highlight that autologous bone graft is the gold standard clinical material for bone regeneration. However, limited availability and donor site morbidity are concerned. Bone allograft becomes the second higher option for orthopaedic procedures due to the availability in various forms and large quantities [22].

Skin transplantation is performed in 2 (20.0%) medical-sanitary institutions with activities in the field of tissues and cells donation and transplantation. Annually there are performed skin grafting surgeries 10.0±1.0 (from 9 up to 11 surgeries) in average (fig. 2), with 71.5±0.5 grafts (from 71 up to 72 grafts) on a surface of 11005.0±358.5 cm^2 (from 10647 up to 11364 cm^2).

Over 1/2 (23 – 57.5%) of surgeons would perform more skin grafting surgeries if those grafts were provided by the tissue bank. All respondents are hired by the republican medical-sanitary institutions.

The amniotic membrane transplantation is performed in 6 (60.0%) medical-sanitary institutions with activities in the field of tissues and cells donation and transplantation. The total annual number of amniotic membrane transplantations in average is of 10.67±6.72 (from 3 up to 44 amniotic membrane grafting surgeries) (fig. 2), with 11.83±6.55 grafts (from 3 up to 44 grafts) on a surface of 559.0±279.16 cm2 (from 45 up to 1814 cm2).

Over 1/2 (39 – 52.7%) of surgeons would perform more amniotic membrane grafting surgeries if those grafts were provided by the tissue bank – in average 7.75±0.75 grafts (from 7 up to 10 grafts). In the private medical-sanitary institutions this parameter is statistically more frequent (6 – 100.0%) compared to the municipal institutions (0 – 0%; p<0.001) and the republican institutions (33 – 53.2%; p<0.001).

Our study showed that musculoskeletal grafts for transplant surgery remain the most requested in our country as in the other countries of the world [5, 9].

Generally, 100 (94.3%) surgeons from the medical-sanitary institutions with activities in the field of tissues and cells donation and transplantation consider that the increasing in number of tissue grafting surgeries performed in their institutions would contribute to improve the patients' life quality. As to the republican medical-sanitary institutions, this opinion is statistically less often (76 – 92.7%), compared to the municipal institutions (11 – 100.0%; p<0.05) and private institutions (13 – 100.0%; p<0.05) (fig. 5).

About 64 (94.1%) surgeons from the medical-sanitary institutions with activities in the field of tissues and cells donation and transplantation declared that enlarging the types of tissues transplanted is a very necessary measure. This opinion is also shared (p>0.05) by respondents from the republican (55 – 94.8%), municipal (4 – 80.0%) and private (5 – 100.0%) institutions.

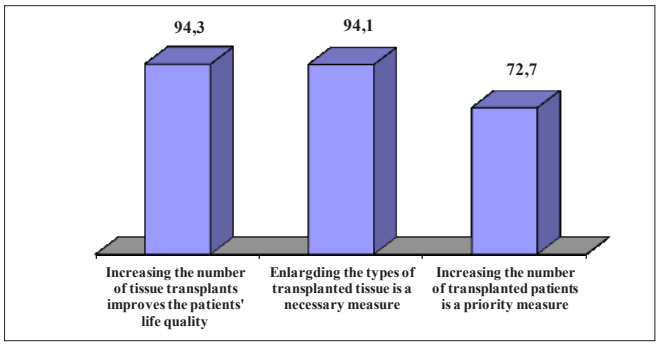

Fig. 5. Opinion of surgeons from the medical-sanitary institutions with transplant activities regarding the perspective of tissues and cells transplantation (%).

Over 2/3 (117 – 72.7%) of surgeons from all medical-sanitary institutions participating in the survey consider

that increasing the number of transplanted patients in the next 5 years is a priority for the institution management. This opinion is also shared by surgeons from the institutions with activities in the field of tissues and cells donation and transplantation (80 - 75.5% of respondents) and from the institutions without the activities in this field (37 – 67.3% of respondents; p>0.05).

Despite this, only about 2/3 (68 – 64.2%) of respondents plan in the next 5 years to extend the types of tissue and cell grafting (other than those transplanted currently): 5 (45.5%) respondents from the municipal institutions, 5 (38.5%) respondents from the private institutions and 58 (70.7%) respondents from the republican institutions (fig. 6).

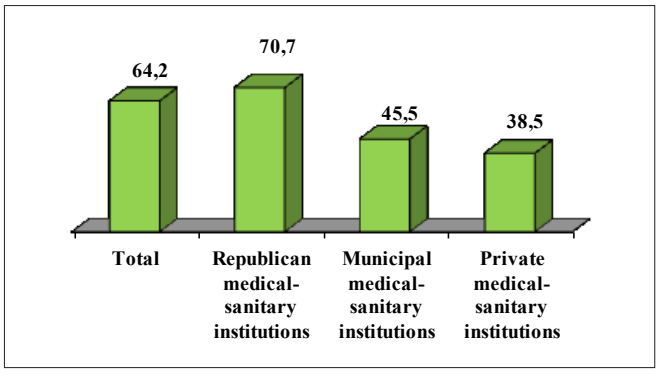

Fig. 6. Surgeons' plan to extend the types of tissues and cells transplanted over the next 5 years by institution level (%).

This expectation is statistically more frequent among surgeons from the republican medical-sanitary institutions – 58 (70.7%) respondents compared to surgeons from the private institutions – 5 (38.5%) respondents (p<0.05).

The assessment of opinion of surgeons regarding the dynamic of providing those institutions with tissue grafts over the past 5 years found that 81 (76.4%) respondents confirmed that situation has improved and 25 (23.6%) respondents consider that it has not changed. Depending on the level of institution, the following results were achieved: 64 (78.0%) respondents from the republican medical-sanitary institutions, 11 (100.0%) respondents from the municipal institutions and 6 (46.2%) respondents from the private institutions consider that situation in providing the institution with tissue grafts over the last 5 years has improved; 18 (22.0%) respondents from the republican institutions and other 7 (53.8%) respondents from the private institutions consider that situation with providing grafts has not changed over the past 5 years. Statistically significant differences in those opinions as to tissue grafts provided to the institutions were noted in respondents from the municipal and private institutions (p<0.01), in respondents from the municipal and republican institutions (p<0.01) and in respondents from the private and republican institutions (p<0.05) (fig. 7).

About 2/3 (66 – 62.3%) of surgeons from the medical-sanitary institutions with activities in the field of tissues and cells donation and transplantation declare that the number of patients needing transplant has increased, 38 (35.8%)

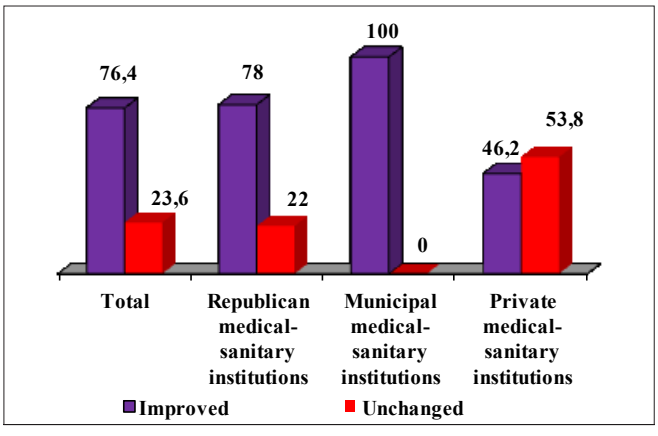

Fig. 7. The opinion of surgeons regarding the dynamic of providing with tissue grafts during the last 5 years depending on the level of the institution (%).

respondents consider that the number of patients needing transplant has unchanged and only 2 (1.9%) respondents mentioned that the number of patients needing transplant has decreased. As many as 51 (62.2%) respondents from the republican institutions, 9 (81.8%) respondents from the municipal institutions and 6 (46.2%) respondents from the private institutions revealed that number of patients who need grafting has increased, 29 (35.4%) respondents from the republican institutions, 2 (18.2%) respondents from the municipal institutions and 7 (53.8%) respondents from the private institutions declared that the number of patients in need of a transplant has remained unchanged and only 2 (2.4%) respondents from the republican institutions noted that the number of patients who need transplant has decreased. Differences in those opinions could not be set statistically significant (fig. 8).

Therefore, priority in establishing an efficient tissue and cell transplantation system should be given to: promoting the training of specialists based on the best experiences, endowing the medical-sanitary institutions with adequate medical equipment, establishing an efficient system for identifying persons that could become tissue donors post-mortem and studying how to encourage donors alive to donate [11, 16, 23, 24].

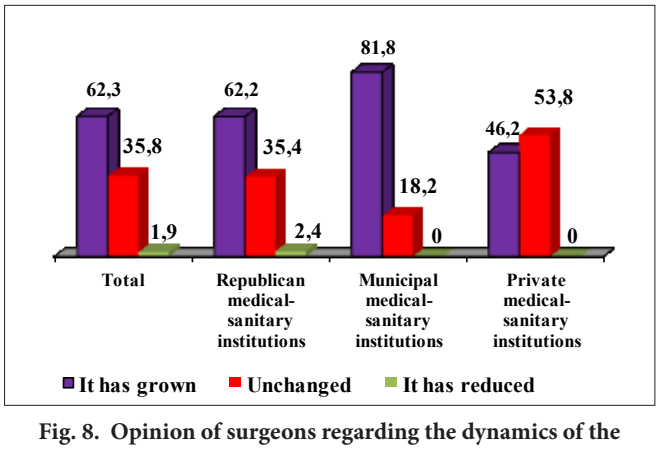

Fig. 8. Opinion of surgeons regarding the dynamics of the number of patients requiring transplantation according to the institution level (%).

Conclusions

1. The estimation of surgeons' opinion regarding the clinical needs for tissues and cells revealed the following: 59.4% of respondents consider that their institutions are sufficiently supplied with grafts, 21.7% of respondents consider that their institutions deal with exceptional cases of lack of grafts, 13.2% respondents consider that the grafts are sufficient for urgent patients and 5.7% respondents consider that grafts are enough only for patients in emergency need.

2. About 44.7-57.5% of surgeons from the medical-sanitary institutions with activities in the field of tissues and cells donation and transplantation would transplant more grafts if those grafts were provided by the tissue bank, and 64.2% of surgeons plan to extend the types of transplanted tissues and cells (other than those transplanted currently) over the next 5 years.

3. About 94.3% of surgeons from the medical-sanitary institutions with activities in the field of tissues and cells donation and transplantation consider that the increasing in number of tissue transplants performed will contribute to improve the patients' life quality, 94.1% of surgeons declare that enlarging the types of tissues transplanted is a necessary measure, and 72.7% of surgeons are convinced that increasing the number of transplanted patients over the next 5 years is a priority measure in the management of the institution they operate.

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Author's ORCID iD and academic degrees
Tatiana Timbalari – https://orcid.org/0000-0003-1391-9107, MD.

Author's contribution
TT conceptualized the idea, conducted literature review, wrote the manuscript, revised and approved the final text.

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The trial was the author's initiative. The author is independent and takes responsibility for the integrity of the data and accuracy of the data analysis.

Ethics approval and consent to participate
The study was approved by the Ethic Committee of *Nicolae Testemitsanu* State University of Medicine and Pharmacy, Chisinau (protocol No 2, 27.10.2016).

Conflict of Interests
No competing interests were disclosed.

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Prevalence of overweight in adults in the Republic of Moldova

***Angela Tomacinschii, Oleg Lozan**

School of Public Health Management, *Nicolae Testemitsanu* State University of Medicine and Pharmacy Chișinau, the Republic of Moldova

Authors' ORCID iDs, academic degrees and contributions are available at the end of the article

Corresponding author: angelatomacinschii@gmail.com

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Abstract

Background: Preventing and decreasing overweight becomes a priority for public health both, worldwide and the Republic of Moldova is not an exeption. The phenomenon of overweight is not deeply studied in the country so far. The latest data available are reflected in the national research on the prevalence of risk factors for non-communicable diseases in the Republic of Moldova, where it is stated that about 50% of the adult persons (18 years and over) suffer from excess weight. In this context, it was proposed to determine the prevalence of excess weight among adults over 18 years in the Republic of Moldova and to estimate the risk of overweight depending on demographic factors.

Material and methods: Descriptive, transvers study (cross-sectional study) based on primary data collection was carried out. 1200 adults over 18 years participated at this study. Statistical analysis of the data involved: frequency analysis, group comparisons, estimation of risk associated with excess weight OR (odds ratio) according to the geographical area, residence area, age group and gender. Data interpretation was performed on the basis of statistical significance ($p < 0.05$) at the 95% confidence interval. According to the WHO, excess weight (overweight) is considered when BMI values are equal to or greater than 25.0 kg / m2.

Results: The results of the research reveal significant differences of excess weight depending on the age, sex and geographical areas. The highest prevalence of excess weight was observed among persons over 40 years (74.7%), women (57.9%), residents of the Center area (62.2%) and of the rural area 62.4%.

Conclusions: The prevalence of excess weight in the Republic of Moldova constitutes 57.6% among the adult population, with the predominance among persons over 40 years of age, women and inhabitants of the Center area and the rural area.

Key words: overweight, demographic factors, Moldova.

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Introduction

Overweight becomes one of the most challenging public health problems and is considered one of the most important causes of morbidity and mortality among the adults [1-3]. According to the data of the World Health Organization (WHO), more than 1.9 billion adults (over 18 years) suffer from overweight and more than 650 million – from obesity [4, 5]. According to several studies, overweight and obesity are the basic factors that induce the development of various mental and physical disorders [6, 7]. Overweight is considered the sixth risk factor, which contributes to the global burden of the diseases around the world, and obesity – the fifth risk factor for mortality worldwide [8]. The data available in the scientific literature highlight the increasing prevalence of overweight, including a financial burden on health services and on the economy as a whole [9]. In addition to the physical and mental health problems caused by this, the impairment of the productivity aspect is emphasized, and also the economic and financial ones [2].

The latest data available are reflected in the national research on *The prevalence of risk factors for non-communicable diseases in the Republic of Moldova* [10], where it is stated that about 50% of the adult persons (18 years and over) suffer from excess weight. In this context, the problem of excess weight is becoming a top priority for the public health of the Republic of Moldova, and the in-depth study of the field should provide practical evidence that would guide the supplementation of existing policy documents in order to reduce this phenomenon.

In this context, it was proposed to determine the prevalence of overweight among adults over the age of 18 in the Republic of Moldova and to estimate the risk of overweight depending on demographic factors.

The results of the study will serve as practical evidence for guiding and supplementing the existing policies in order to reduce the phenomenon of excess weight in the Republic of Moldova.

Material and methods

The present study is descriptive, cross-sectional based on primary data collection. Inclusion criteria for the study were: 1) being over 18 years of age; 2) resident of the Republic of Moldova; 3) consent to participate in the study; 4) mental ability to answer questions. The sample of the study was calculated on the basis of selective irrevocability, picked out by proportional stratification depending on

the geographical areas of the country and comprised 1200 people. The primary data were collected on the basis of the interview, structured in the questionnaire, accompanied by informed consent. In order to ensure the confidentiality of the data, the information collected was depersonalized and codified. The statistical analysis was performed using SPSS / Statistical Package for Social Sciences for Windows/ version 20. The quality of the data was ensured by: Visual Verification and validation of the data from the completed questionnaires and automated validation through the SPSS program. Geographical areas were considered: North (without Balti municipality), Center (without Chisinau municipality) and South. The body mass index (BMI) was calculated using the formula: BMI = G (kg) /T²(m²), where G – is body weight, T-weist. According to the international classification of BMI [5, 11, 12], the sample was grouped into two categories, BMI≤24.9 kg/m² (not overweight) and BMI≥25.0 kg/m² (overweight or obese). Statistical analysis of data involved: frequency analysis, group comparisons were made by using the "T"-independent test for continuous variables and X2-Pearson or Fisher for categorical variables. For risk estimation, the OR (odds ratio) was used. Data interpretation was based on statistical significance (p<0.05) at 95% confidence interval.

Results

Out of 1200 adults surveyed more than half (56.5 ± 1.43%) were from rural areas, women constituted 52.8 ± 1.44% and 59.4 ± 1.41% were persons over 40 years old. The geographical distribution comprised 33.1±1.35% respondents from the North area; in 40.3±1.42% – from the Center area; in 26.6±1.28% – from the South area (p<0.05).

The average BMI among the studied population was 27.3 [95% CI: 26.9-27.5]. The average BMI value, depending on the residence area of the study population, was estimated to be equal to 27.0 [95% CI: 26.6-27.4] in the urban area, with the minimum and maximum values between 14.2 and 44.9 and being equal to 27.6 [95% CI: 27.1-28.1] in rural areas, with the minimum and maximum values between 15.2 and 46.4 (p> 0.05).

Out of the studied population 57.6±1.43% were overweight. Most of the persons over 40 years were overweight (74.7% ± 1.67%) (p <0.005). The prevalence of overweight among women was – 57.9 ± 1.70%. It was estimated that persons in the age group of adults over 40 years were 2.1 (p <0.001) and 1.1 (p <0.05) times more likely to be overweight. Regarding the dependence factor on residence area, the research showed that 62.4 ± 1.40% of the persons in the rural area were overweight, (compared to 54.2% in the urban area), therefore, the residence factor was rated as an important factor for overweight (OR = 1.0; p <0.001).

The analysis of the prevalence of excess weight depending on the geographical areas revealed the prevalence of excess weight in the Center area (62.2 ± 2.59%). It was estimated that the residents of the Center area are 1.3 times more likely to become overweight (p <0.005) (tab. 1, fig. 1).

Table 1

Estimating the risk of overweight depending on demographic factors

Name	BMI ≤25.0 kg/m²	OR (II95%)	P-Value
	abs. (%)		
Age group			
18-39 years old	35.4	1	
40 y.o. and over	74.7	2.1 (1.9- 2.4) 2.1 (1.9- 2.4)	p<0.001
Residence environment			
Urban	54.2	1	
Rural	62.4	1.1 (1.0-1.3)	p<0.01
Sex			
Male	56.8	1	
Female	57.9	1.3 (1.1-1.8)	p<0.05
Geographical distribution			
Area			
North	59.9	1.2 (0.8-1.7)	p>0.05
Centre	62.2	1.3 (1.1-1.8)	p<0.05
South	55.9	1	
Total per areas	59.7	1.4 (1.1-1.8)	p<0.05
TOTAL	57.6		

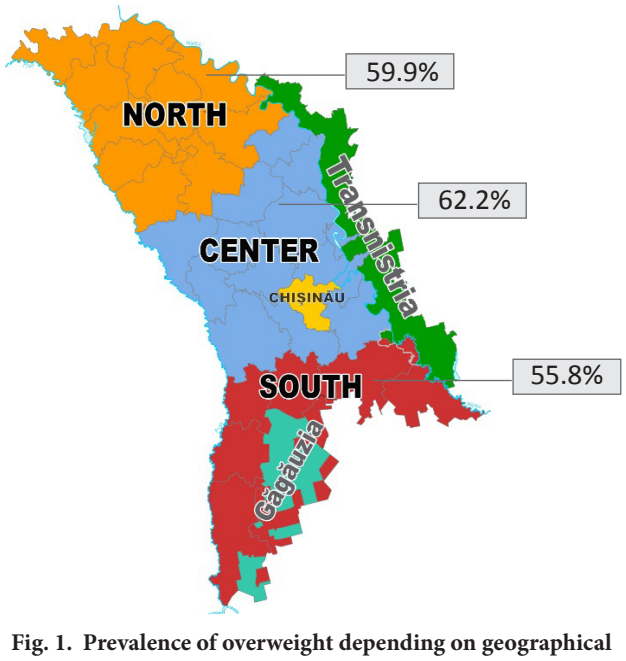

Fig. 1. Prevalence of overweight depending on geographical areas.

Discussion

For the first time, in the Republic of Moldova, the prevalence of excess weight was analyzed depending on the residence environment, geographical areas and demographic factors as risk factors for overweight.

This research examined the prevalence of overweight among adults in the Republic of Moldova and established a prevalence of 57.6%. At the same time, the latest data currently available in the country, reflected by the National

research, stated that about 50% of adult people are overweight or obese [10]. The average BMI values for the entire population under research, depending on the residence and sex environment were similar to those assessed by the National study. The age of over 40, rural residence, Center area and female gender were assessed as a risk factor for excess weight. This has been investigated and documented through studies in other countries as well [13-16].

The difference in the prevalence of excess weight depending on the geographical areas requires further study to assess the specific and predominant factors causing this phenomenon.

Conclusions

1. The prevalence of excess weight among adults over the age of 18 in the Republic of Moldova constitutes 57.6%, which shows that overweight is a major public health problem.

2. Research has shown that overweight is prevalent among people over the age of 40 (74.7%), and according to sex, women are more likely to become overweight compared to men.

3. The analysis of the prevalence according to the area of residence indicates that 62.4% of the persons with excess weight come from the rural area, therefore the area of residence was considered an important factor for the excess weight.

4. The research highlighted statistical differences regarding the prevalence of excess weight depending on the geographical areas North, Center, South. Thus, according to the geographical areas of the Republic of Moldova, the results showed an overall prevalence of weight gain of 62.2% in the Center area, 59.9% – in the North area and 55.8% – in the South area.

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Authors' ORCID iDs and academic degrees

Angela Tomacinschii – https://orcid.org/0000-0003-4864-1766, MD.
Oleg Lozan – https://orcid.org/0000-0002-1552-1496, MD, PhD.

Authors' contributions

AT conceptualized the project and drafted the first manuscript. OL interpreted the data and critically revised the manuscript. Both authors approved the final version of the manuscript.

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Ethics approval and consent to participate

No approval was required for this study.

Conflict of Interests

No competing interests were disclosed.

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Umbilical cord coiling abnormality as a predictor of maternal and fetal outcomes

Alina Alsatou

Department of Obstetrics and Gynecology, *Nicolae Testemitsanu* State University of Medicine and Pharmacy Chisinau, the Republic of Moldova

Author's ORCID iD, academic degrees and contributions are available at the end of the article

Corresponding author: linka-05@mail.ru

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Abstract

Background: The umbilical cord forms connecting link between the fetus and placenta through which the fetal blood flows to and from the placenta. Its three blood vessels pass along the length of the cord in a coiled or helical fashion (spiral course). The aim of the study was to evaluate umbilical cord coiling abnormalities and determine its relationship with maternal and perinatal outcomes.

Material and methods: The study included 190 patients divided into 2 groups: L_1 – 95 patients with UC abnormalities and L_0 – 95 with normal UC. The umbilical cord index was measured after delivery of the adnexal complex, which was defined as the total number of coils divided by the total length of the cord in centimeters.

Results: The hypo- and hypercoiling umbilical cord suggests the high risk of fetal distress ($p<0.0001$), instrumental vaginal deliveries, the admission of the newborn in the neonatal intensive care ($p<0.0001$) and perinatal morbidity, which demanded a transfer to other medical facilities ($p<0.05$). UC torsion was associated with insufficiency of placental circulation, IUGR, fetal hypoxia and fetal mortality ($p<0.05$). The straight cord had significant correlation with maternal infections, antenatal mortality and preterm labor in anamnesis, placental insufficiency, IUGR and neonatal morbidity ($p<0.05$).

Conclusions: Umbilical coiling index was found to be an important predictor of adverse maternal and perinatal outcomes. To conclude, abnormal umbilical coiling index is associated with an increased rate of adverse antenatal and neonatal features. The association shows wide variations in the numerous studies done so far.

Key words: umbilical cord, coiling abnormality, straight cord, perinatal outcome.

Cite this article

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Introduction

Umbilical cord (UC) is a vital structure which acts as a conduit between the developing fetus and placenta [1]. It carries nutrients, oxygen and fluids necessary for the intrauterine life. This unique lifeline needs optimal protection which is provided by Wharton's jelly, amniotic fluid, helical patterns and coiling of the umbilical vessels [2, 3].

The origin of umbilical cord coiling is unknown. Hypotheses include fetal movements, active or passive torsion of the embryo, differential umbilical vascular growth rates, fetal hemodynamic forces, and the arrangements of muscular fibers in the umbilical arterial wall [4].

Of the many characteristics of the human umbilical cord, a most mysterious and intriguing one is the twisted or spiral course of its component blood vessels. Mathematically speaking, the vessels of the cord are wound as cylindrical helices, rather than spirals, but both terms are used interchangeably to avoid confusion [3]. The coiling of the umbilical vessels develops as early as 28 days after conception and is present in about 95% of fetuses by 9 weeks of conception. The helices may be seen by ultrasonographic examination as early as during the first trimester of pregnancy [5]. Many investigators have found that majority of the cords have a left-sided twist [6-8].

The total number of coils seen is between 0 and 40. The coiled umbilical cord perhaps of its elastic properties, is able to resist external forces that might compromise the umbilical vascular flow. Umbilical coiling appears to confer turgor to the umbilical unit, producing a cord that is strong, yet flexible [9]. *A coil* is of 360-degree spiral course of umbilical vessels. Umbilical cord index (UCI) is defined as the total number of coils divided by the total length of the cord in centimeters [10]. Although UCI can be calculated antenatally by ultrasonography, limited data is available as to its accuracy.

Thus, umbilical cord is vital to the development, well-being, and survival of the fetus, yet this is vulnerable to kinking, compressions, traction, and torsion which may affect the perinatal outcome [11]. Umbilical cord abnormalities are among the pathologies which are still not clarified for the factors in their etiologies, do not have sufficient data on their diagnosis and treatment but may lead to severe perinatal complications. The present study has been undertaken to compare the maternal and perinatal outcome with the abnormal coiling of the cord with respect to umbilical coiling index.

Material and methods

This prospective study was carried out in the Department of Obstetrics and Gynecology at the clinical base of Munici-

pal Clinical Hospital No 1, *Nicolae Testemitsanu* State University of Medicine and Pharmacy. The study and the control groups included 95 patients each (with (L_1) or without (L_0) umbilical cord abnormalities), as determined by sample size calculations.

Umbilical cord assessment was based on macroscopic examinations for length, diameter, color, insertion site, number of twists and vessels. Immediately after delivery, the umbilical cord was clamped at the fetal end and cut with scissors taking care not to milk the cord (as the latter might affect the umbilical cord index). The length of the cord from the fetal end to the placental insertion was measured with a tape (in centimeters). A coil was taken as one complete 360-degree spiral course of the umbilical vessels, and the total number of these complete vascular coils of the entire cord was determined. The generally accepted method of assessing the degree of the umbilical cord coiling is by calculation of the umbilical coiling index (UCI), defined as total number of complete vascular coiling per total length of cord in cm.

Umbilical cord abnormalities (UCA) included in the study were: normocoiled cord (1-3 coils per 10 cm), hypercoiled cord (more than 3 coils per 10 cm), torsion cord (more than 5 coils per 10 cm), hypocoiled cord (fewer than 1 coil per 10 cm), straight cord (the entire length of the umbilical cord shows no evidence of coiling).

The inclusion criteria in the research were: gestational age between 22^{+0}–41^{+6} weeks, spontaneous and singleton pregnancy, maternal age ≥ 18 years, research participation agreement. The exclusion criteria were: gestational age ≤ 21^{+6} weeks and ≥ 42^{+0} weeks, pregnancy, which occurred as a result of assisted reproduction technologies, multifetal gestation, congenital malformations of the fetus, decompensated somatic pathology of the patient, age of the patient ≤ 18 years, patients who refused voluntary participation in the research.

Clinical details obtained along with the cord specimens were: maternal age, gravidity and parity, gestational age, medical conditions complicating pregnancies, the status of the amniotic fluid, perinatal complications, anthropometric parameters of the newborn, APGAR score at 1 and 5 minutes as recorded by the obstetricians. All the mothers and babies were followed up till discharge.

Statistical analysis was performed using Statistical Package for Social Sciences for Windows (SPSS Version 23.0), Statistical Analysis System (SAS Version 9.4) and Microsoft Excel 2016. The significance was tested by using a Chi-square test, the Phi coefficient, the contingent coefficient, the Cramer V coefficient, and the Fisher's exact test. For all quantitative characteristics in the compared groups were evaluated the arithmetic means and mean-square (standard) errors of the mean, coefficient of variation, median, mode, and quartiles. *P* value of less than 0.05 was regarded as statistically significant.

Results and discussion

The age of the patients included in the control group (L_0) was between 19-37 years with the average of 27.86±4.36 years and in the study group (L_1) was between 19-40 years with an average of 29.09±4.84 years (fig. 1).

The rate of respondents living in the urban areas of the country was 87.37% (83) vs 82.11% (78) in the control and study groups respectively (p=0.31) (fig. 2). The analysis of the marital status in the study groups demonstrated 85.26% (81) married women in the L_0 vs 77.89% (74) in the L_1 (p=0.30) (fig. 3). Thus, according to the age criterion, living environment, marital status, the examined lots were homogeneous.

The birth weight in the control group (L_0) was 2460–4780 g (mean 3470.21±463.80 g); in the study group (L_1) – 1030-4760 g (mean 3198.11±673.68 g). The newborn length in the L_0 = 45-57 cm (52.37±1.99 cm) vs 34–58 cm (51.06±3.63 cm) in the L_1. APGAR score at 1 min L_0 = 7-10 points (8.66±0.69) vs L_1 = 0-9 points (7.46±1.65). APGAR score at 5 min L_0 = 8-10 points (9.13±0.67) vs L_1 = 0-10 points (8.01±1.7). The length of the umbilical cord in the L_0 ranged between 42-69 (55.6 ± 7.29) cm, but in the L_1 = 25-90 (56.46 ± 17.23) cm.

In the study group was determined hypocoiled cord – 35.79% (34), hypercoiled cord – 7.37% (7), torsion cord – 6.32% (6) and straight cord – 15.79% (15).

Umbilical cord coiling abnormality was significantly associated with adverse maternal and perinatal outcome. Both hypo- and hypercoiling groups were found to be significantly associated with intrapartum fetal heart rate abnormalities which suggest the high risk of fetal distress (p<0.0001) and instrumental vaginal deliveries (vacuum extraction (p<0.0001). This is explained by the fact that coil-

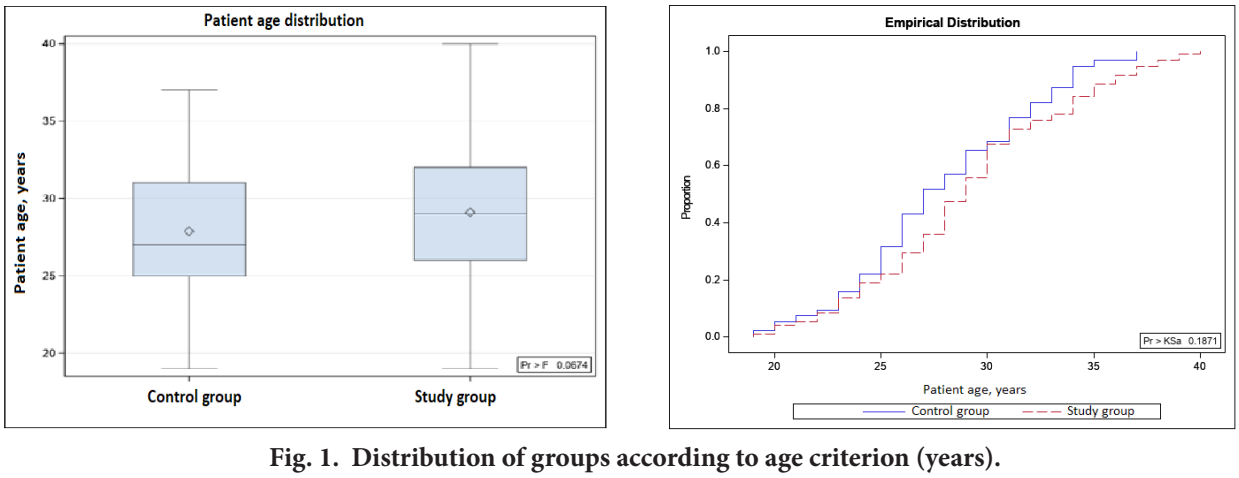

Fig. 1. Distribution of groups according to age criterion (years).

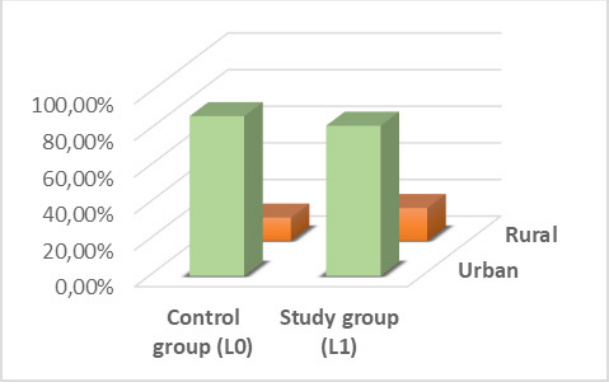

Fig. 2. Distribution of groups according to the living environment.

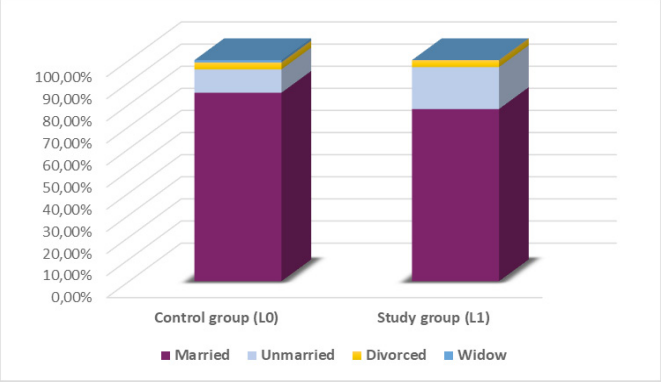

Fig. 3. Distribution of groups according to the marital status.

Table 1

Maternal and perinatal factors of the umbilical cord coiling abnormalit

The evaluated characteristics	Chi-square test (χ^2)	*df*	*P value*	*Cramer's V*
Hypocoiled UC				
Insufficiency of placental circulation (IB, II)	40.93	9	<0.0001	0.27
Fetal bradicardia	51.09	6	<0.0001	0.37
Pathological CTG	53.95	6	<0.0001	0.4
Vacuum extraction	21.25	3	<0.0001	0.37
Fetal hypoxia	56.02	3	<0.0001	0.55
Pathological adaptation period	22.11	3	<0.0001	0.34
Neurological disorders of the newborn	9.31	3	0.02	0.34
Hypercoiled UC				
Harmful factors at work	9.71	3	0.02	0.3
Intrauterine infection (chorioamnionitis)	27.6	3	<0.0001	0.38
Fetal bradicardia	51.09	6	<0.0001	0.37
Induction of labor	10.27	3	0.01	0.25
Labor Weakness	40	9	<0.0001	0.3
Vacuum extraction	21.25	3	<0.0001	0.37
Pathological adaptation period	22.11	3	<0.0001	0.34
Neonatal morbidity	19.78	3	0.0002	0.32
Transfer to the second stage *or* other medical facilities	15.50/16.8	3	0.0014/0.0008	0.28/0.3
Torsion UC				
Antenatal mortality in anamnesis	30.82	3	<0.0001	0.4
Insufficiency of placental circulation (IA)	16.38	3	0.0009	0.3
IUGR	25.74	3	<0.0001	0.36
Fetal hypoxia	56.02	3	<0.0001	0.55
Stillbirth	9.47	3	0.02	0.22
Straight UC				
TORCH-infection (Mycoplasma, CMV)	42.4	24	0.01	0.27
Antenatal mortality in anamnesis	11.72	3	0.008	0.25
Preterm labor in anamnesis	8.24	3	0.04	0.2
Placental insufficiency (IB, II)	40.93	9	<0.0001	0.27
IUGR	8.04	3	0.04	0.31
Congenital pneumonia of the newborn	11.11	3	0.01	0.37

Note: UC – umbilical cord, CTG – cardiotocography, IUGR – Intrauterine growth restriction, CMV – Cytomegalovirus.

ing provides turgor and compression resistant properties to the cord which become compromised as the cord becomes hypocoiled [12].

In our study torsion and straight cords were found to be significantly associated with IUGR (p<0.0001 and 0.04). Ankita M. et al. and Agarwal S. et al. [5, 13] demonstrated a significant association between IUGR babies and hypercoiling cord (p=0.000 and p=0.0323). Ezimokhai et al. and Monique de Laat et al. obtained a similar result in their studies [14, 15]. However, Machin et al. [16] found IUGR to be associated with hypocoiling. They summarized that since adequate coiling prevents compression of the cord, hypocoiling in the long run results in reduced fetoplacental circulation, thus resulting in growth restriction.

Another interesting aspect to be analyzed is the admission of the newborn in the neonatal intensive care unit (NICU).

Dr. T. Shobha et al. [12] demonstrated that the newborn with hypocoiled and hypercoiled cords required NICU care, with strongly significant suggestive of correlation between the two groups (*p* value being 0.001 and 0.0136 respectively). We obtained a similar result that suggests strong correlation between hyper- and hypocoiled cords (p<0.0001).

In case of the torsion of umbilical cord, blood flow decreases below the critical level and this leads to fetal hypoxia, intrauterine growth restriction (IUGR) and fetal death (p<0.05). Akgün N. et al. and Yuce T. et al. confirm these results [17, 18].

No association was found between abnormal coiling and extragenital maternal diseases, pregnancy induced hypertension, oligo(poly)hydramnios, obstetric hemorrhage, premature rupture of membranes, meconium staining of liquor (p>0.05). Ezimokhai et al. [14] found hypercoiling to be associated with extremes of maternal age (< 20 and > 35 years). None of the other studies found age to be a significant factor [14].

The relation between UCI and various maternal/perinatal outcomes has been summarized in table 1. To conclude, abnormal umbilical coiling index is associated with several adverse antenatal and neonatal features.

Conclusions

1. The hypocoiling umbilical cord suggests the high risk of fetal distress (p<0.0001), instrumental vaginal deliveries (vacuum extraction), the admission of the newborn in the neonatal intensive care (p<0.0001) and neurological disorders of the newborn (p=0.02).

2. The hypercoiling umbilical cord was correlated with intrauterine infection (chorioamnionitis), labor weakness, fetal distress, pathological adaptation period, neonatal morbidity, which demanded a transfer to other medical facilities (p<0.05).

3. The torsion of umbilical cord was associated with insufficiency of placental circulation, IUGR, fetal hypoxia and fetal mortality (p<0.05).

4. The straight cord had significant correlation with maternal infections, antenatal mortality and preterm labor in anamnesis, placental insufficiency, IUGR and neonatal morbidity (p<0.05).

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Author's ORCID and academic degrees
Alina Alsatou – https://orcid.org/0000-0003-0126-2275, MD, PhD.

Author's contributions
AA designed the trial and interpreted the data, drafted the first manuscript, revised and approved the final version of the manuscript.

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Heart rate variability in people with borderline type personality

[*1]Svetlana Lozovanu , [1,2] Ion Moldovanu, [1]Victor Vovc, [1]Tudor Besleaga, [1]Andrei Ganenco

[1] Department of Human Physiology and Biophysics, *Nicolae Testemitsanu* State University of Medicine and Pharmacy
[2]Department of Headache and Vegetative Disorders, Institute of Neurology and Neurosurgery
Chisinau, the Republic of Moldova

Authors' ORCID iDs, academic degrees and contributions are available at the end of the article

*Corresponding author: svetlana.lozovanu@usmf.md

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Abstract

Background: Reduced HRV is associated with a variety of conditions such as diabetic neuropathy, sepsis, myocardial infarction, but lately it has gained increased interest in psychiatry due to the connection between autonomic dysfunction and psychiatric pathologies. Borderline personality disorder (BPD) with an increased rate of cardiovascular mortality, and characterized by emotional instability, is ideal for studying heart rate variability.
Material and methods: 203 subjects were initially evaluated with Personality Inventory for DSM-5, PID-5, (DSM-5 – Diagnostic and Statistical Manual of Mental Disorders, 5th edition), and 2 groups have been selected: control group that included 69 subjects and borderline personality disorder (BPD) group that included 34 subjects. Heart rate variability (HRV) was analyzed from an electrocardiography signal, recorded in 3 conditions: resting, pain stimulation, period following the pain stimulation.
Results: In post-pain period, in subjects with BPD, the HRV parameters indicate an increase of sympathetic influences on heart rate and a reduction of vagal modulatory effects. The values in these subjects did not return to the initial values in the post-pain period as they did in the control group, but, on the contrary, the accentuation in the dynamics of the sympathetic influence was registered, even compared to the pain period.
Conclusions: Subjects with BPD presented an increased vagal modulation at rest, which was reduced during pain stimulation and did not return rapidly to the initial value after removing the painful stimulus, which can be proof of the inertia of autonomic influences in these subjects.
Key words: borderline personality disorder, heart rate variability.

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Introduction

Heart rate variability (HRV) is the time variation of the intervals between each heartbeat, recorded as RR intervals of the ECG signal. It is a complex physiological phenomenon that results from the change of heart rate by respiratory, circulatory, autonomic, endocrine and mechanical factors. Reduced HRV is associated with a variety of conditions such as diabetic neuropathy, sepsis, myocardial infarction, but lately it has gained increased interest in psychiatry due to the connection between autonomic dysfunction and psychiatric pathologies [1]. Changes in HRV have been reported in many mental disorders [1, 2] as well as correlations of HRV with psychological dimensions such as social cognition [2, 3], interpersonal relations [4, 5] and emotional regulation [6, 7] have been described. The increased incidence of cardiovascular disease associated with psychiatric pathologies has also led to increased attention to the autonomic nervous system. Borderline personality disorder (BPD) with an increased rate of cardiovascular mortality, and characterized by emotional instability, is ideal for studying heart rate variability. Changes in the heart rate occur due to a constant need of the heart to adapt to changing circumstances and it is believed that the loss of balance between the sympathetic and parasympathetic nervous system causes alteration of the HRV structure [8]. In this context, HRV is a measure of autonomic nervous system balance, and therefore may provide a quantification of the physiological changes associated with mental illness.

In the last two decades since the first studies appeared and until now, multiple connections between resting heart rate variability and psychological functions, including psychopathological expression, have been described. In general, these early studies, and many of those that appeared afterwards, showed that HRV correlates with various adaptive psychological effects among children, adolescents, and adults, including the empathic response to other sufferings [9, 10], social competence [5], ability to hold attention for a long time [11], cognitive performance [12], adjustments behavior during social challenges and positive interactions with partners [13]. Low HRV at rest, or a large reduction associated with various challenges (particularly emotions) are associated with symptoms of both introverted and extroverted psychopathology [6, 14-16], with a broad spectrum of psychopathological syndromes, including anxiety [12,

17], phobias [14], attention deficit [18], emotional insensitivity [6, 8, 18], behavior disorder [14, 15], depression [17], non-suicidal self-injury [17, 19], panic attacks [5, 7, 17]), hostility [12, 17, 19], psychopathy [12], schizophrenia and others. In addition, internalizing or externalizing comorbid symptoms predict an additional reduction in cardiac variability during emotional challenges than internalizing or externalizing symptoms of a self-esteem [17]. This impressive long list suggests that HRV at rest and low reactivity to emotional challenges mark one or more essential self-regulatory functions that are disturbed by various forms of psychopathologies. Understanding the neural bases of HRV, and determining the neural bases that give rise to plasticity phenomena, can therefore have important treatment implications, and is a key moment in the path to modifying circuits to reduce the adverse effects on mental health in vulnerable individuals.

BPD is characterized by personality traits in the field of negative affectivity, emotional lability, anxiety, separation insecurity or depression and behavioral characteristics such as disinhibition (including impulsivity and risk-taking) or antagonism (hostility) [20].

This represents a pervasive pattern characterized by instability in interpersonal relationships, self-image and affectivity, as well as by increased impulsivity, which begins at the young adult age, manifests through identity disorders, recurrent suicidal behavior, irritability or anxiety, sustained efforts to avoid a real or imaginary abandonment, chronic feeling of inner emptiness etc. The median prevalence in the population of BPD was estimated at 1.6%, but may reach 5.9%. Taking into account the increased prevalence of BPD of 6% in primary health care and up to 20% in specialized psychiatry centers [21] and the considerable deficiencies caused to patients, the study of heart rate variability could offer to physicians, especially those at primary level, an alternative to pharmacological treatment, by correcting the psychophysiological mechanisms that lead to the appearance of systemic dysfunctions.

Genetic researches on BPD have mainly focused on genes involved in serotoninergic and dopaminergic systems. Tryptophan hydroxylase is an enzyme involved in the 5-HT synthesis of the tryptophan amino acid. Most studies have shown that two isoforms of tryptophan hydroxylase (TPH-1 and TPH-2) are associated with BPD [22]. Serotonin transporter genes (5-HTT), especially 5-HTTLPR (serotonin-transporter-linked polymorphic region}, are associated with BPD, depressive, anxious and obsessive-compulsive traits, but not with suicidal or self-destructive behaviors. On the other hand, monoamine oxidase A (MAO-A), an enzyme that degrades monoamines, especially serotonin, is involved after its recovery from the synaptic cleft. It has been shown that patients with BPD have a variable number of MAO-A gene repeats different from healthy volunteers [22, 23]. The brain-derived neurotrophic factor (BDNF) involved in neurogenesis, synaptogenesis and regulation of serotonin metabolism, the BDNF Val66Val polymorphism could also play a role in the pathogenesis of BPD [24].

The main features of each personality disorder (PD) are emotional disorders that may manifest themselves in different ways, but nonetheless, they are rooted in neural organization. For example, emotional instability is a basic pattern observed in BPD. Other features of this disorder are the tendency toward suicide, outbursts of intense anger, stormy relationships and identity disorders [19]. All these patterns are related to increased attention or sensitivity to social-emotional indices in interpersonal scenarios, the tendency towards self-referential emotional processing and the mechanisms of unregulated emotional processing. Patients with BPD have great difficulty in moving from the psychic equivalence mode to the pretending mode and often retain their perception as an absolute fact [25].

Studies involving mental health and investigating differences in heart rate variability use numerous time, frequency, or non-linear methods that quantify HRV [15, 26-31].

However, there are no widely recognized standards for measuring and quantifying heart rate variability and the clinical interpretation of many features of variability remains contradictory or unknown. Studies involving the analysis of heart rate variability in mental pathologies have minimal standardization [27, 32-35] especially regarding the analysis of time intervals and the methods of recording ECG, making comparing the results of variability over different time intervals additionally difficult. The variations in the duration of the recordings, the degree of activity of the participants and the methods of data collection may be different. For example, data is usually collected when the participant is at rest; however, the participant's posture, time of day, recent consumption of food or drink and many other factors may contribute to heart rate variability. Stimuli in the form of pictures or exercises can be exposed during recordings, making comparison between studies difficult.

The purpose of the study is to determine the autonomic changes in people with borderline personality disorder by studying the variability of the heart rate both at rest and in the pain test.

Material and methods

The current study was designed to determine autonomic changes in people with borderline personality disorder by studying heart rate variability under the influence of painful stimuli.

203 subjects were initially evaluated, between March 2017 – February 2020, in *Nicolae Testemitsanu* State University of Medicine and Pharmacy at the Department of Human Physiology and Biophysics. All subjects had signed an informed agreement. The exclusion criteria were: acute or chronic cardiac diseases; medications that could influence heart rhythm variability. The psychometric evaluation, which preceded the recording of cardiac parameters, was performed using the Personality Inventory for DSM-5, PID-5 (DSM-5 – Diagnostic and Statistical Manual of Mental Disorders, 5th edition) [36], which is a tool for self-assessment of personality traits, developed by the Ameri-

can Psychiatric Association (AAP) in 2012. PID-5 has been translated into Romanian and validated by a working group consisting of the staff of *Nicolae Testemitsanu* State University of Medicine and Pharmacy and the Department of Headache and Vegetative Disorders within the Institute of Neurology and Neurosurgery, in compliance with the rules of translation, adaptation and validation of the International Test Commission and with the consent of the authors. PID-5 is a questionnaire, containing 220 personality self-report items, used to measure maladaptive personality traits, which are characterized in DSM-5. The answers are selected from a four-point scale, from 0 ("very false or often false") to 3 ("very true or often true"). Thus, PID-5 offers scores evaluated on a scale of 4 points, for the 25 facets. Each facet includes from 4 to 14 elements. These facets correspond to the maladaptive personality traits described in section III of the DSM-5 and are included in the five areas of higher order, also described in section III: negative affect, separation, antipathy, disinhibition and psychosis. The score greater than 2 in a certain number of facets is a quantitative index of one of the 6 types of PD: Antisocial, Borderline, Schizotypal, Avoidant, Obsessive-Compulsive or Narcissistic [20, 37, 38].

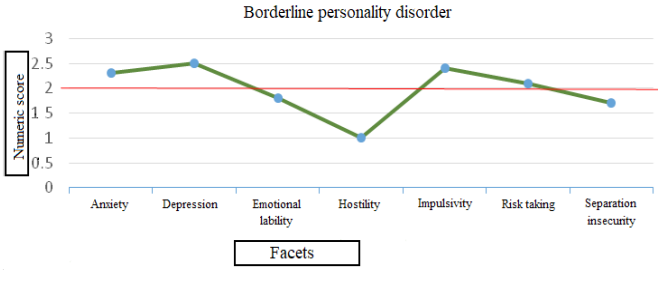

Fig. 1. Subject with Borderline personality disorder, with numerical scores greater than 2 in 4 facets.

Note: for the borderline personality disorder the distribution of the numerical scores of 7 facets is studied. For a positive result, it is necessary that 4 of the 7 facets to have scores greater than 2 on the axis of the ordinates.

Finally, based on the obtained results in the PID-5, the examined subjects were distributed in 2 groups, according to the number of the numeric scores greater than 2 in 4 out of 7 personality traits characteristic for the borderline personality disorder: Anxiety, Depression, Emotional Lability, Hostility, Impulsivity, Risk taking, Separation insecurity:

– First group – PID traits with all numerical score in the range 0-1.99, healthy people (control group);

– Second group – PID: 4 features out of 7 with numerical score more than 2.0, of which at least one to be an obligatory trait (marked in bold) (BPD group).

Subjects with numerical score greater than 2.0 in 1-3 facets of BPD were not included in the study. The BPD profile of a person who was included in the study shows that 4 of the 7 facets have a numeric score greater than 2 (fig. 1). The age of the people included in the study was between 18 and 60 years. The groups were homogeneously distributed according to sex, namely 53 women (mean age 41 years) and 49 men (mean age 39 years). Control group included 69 subjects (N = 69) with a mean age of 40 years (35 women and 33 men) and BPD group included 34 subjects (N = 34) with a mean age of 36 years (18 women and 16 men) (fig. 2, 3).

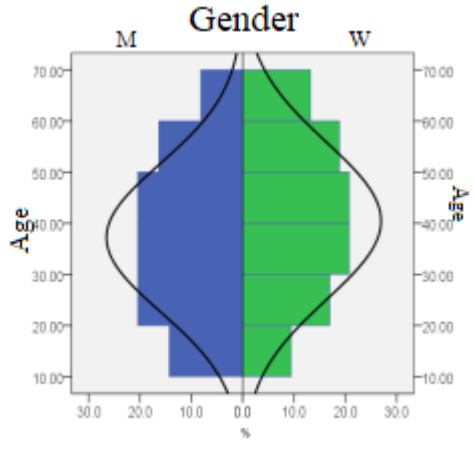

Fig. 2. Distribution of participants according to gender and age.

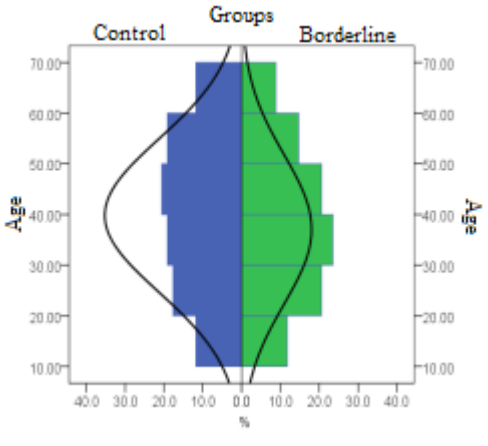

Fig. 3. Distribution of Control subjects and Borderline subjects according to age.

All studies were performed in the morning (08:00-10:00 AM) without the person's consuming any food that morning, in lying position. The experimental protocol included the recording of electrocardiogram in the standard II lead during 3 periods:

1. Breathing at rest (resting period) – in physical, mental and emotional resting conditions, 5 min;

2. Pain test (pain stimulation) – painful stimulus, the cuff of the sphygmomanometer at a constant pressure of 200 mm Hg was applied at the level of the left arm of the subject, for 5 min;

3. Post-pain period – the pressure was removed and recording continued for another 5 min.

The experimental protocol included the recording of ECG in subjects in lying position, in a quiet room, with moderate light, at comfort temperature. During the recording, the subjects were asked to breathe quietly, not to speak and to avoid additional movements.

The ECG signal was recorded using the system Biopac MP-100. The processing of the data was performed with the software "Kubios HRV Standard" (version 3.2.0, 2019), manually removing the artifacts.

The spectral analysis Fourier of the RR interval (NN) variation included the total spectral power TSP (ms^2) and calculation of the spectral components: very low frequency (VLF) – less than 0.04 Hz (ms^2), low frequency (LF) – between 0.04 and 0.15 Hz (ms^2) and high frequency (HF) – more than 0.15 Hz (ms^2). The normalized components LFnu and HFnu were calculated by division of power of components HF (ms^2) and LF (ms^2) by the total spectral power without VLF (ms^2) [32]. LF is often considered as an index of sympathetic modulation, and the HF component is used to evaluate the vagal activity. Following time domain parameters of the HRV were determined:

RMSSD – Root Mean Square of the Successive Differences in neighboring NN intervals (ms);

SDNN – the Standard Deviation of NN intervals during studied period (ms);

NN50 – the number of pairs of successive NNs that differ by more than 50 ms;

pNN50 – pNN50, the proportion of NN50 divided by total number of NNs.

The statistical analysis was performed using IBM SPSS Statistics 23.0 software, t-Student test was used to compare the HRV values inside the groups and between the groups.

Results and discussions

The data about HRV in people with BPD compared to healthy people presented in a review that analyzed HRV in mood disorders, including BPD, in the papers published between 1980 and May 2017 and found in PubMed, PsycINFO, Google Scholar and the Cochrane Library [33] are highly controversial. Our study confirms the heterogeneity of the results obtained by the HRV analysis between the people with BPD and healthy at rest as well as during pain test and after it.

HRV parameters of the time domain method (NN50, pNN50, SDNN and RMSSD) as well as LFnu and HFnu did not show significant differences between the groups included in the study at rest. In the pain test, the differences have been found between the parameters in the frequency domain of the HRV, namely LFnu is 13.9% lower in the BPD group (50.30 ± 3.60) compared to the control group (58.42 ± 2.37), p <0.05; (fig. 4) and respectively HFnu is 19.4% higher in the BPD group (49.45 ± 3.58) compared to the control group (41.39 ± 2.36), p <0.05; (fig. 5).

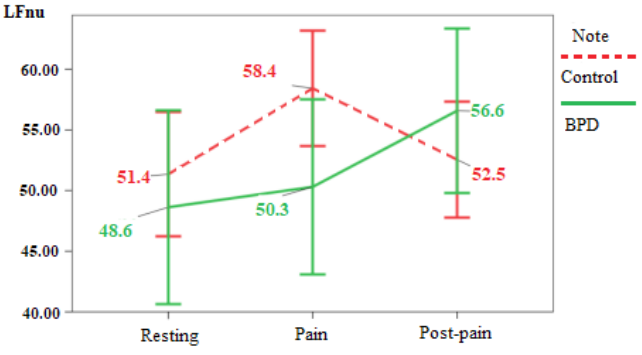

Fig. 4. LFnu values in subjects in control group and BPD group.

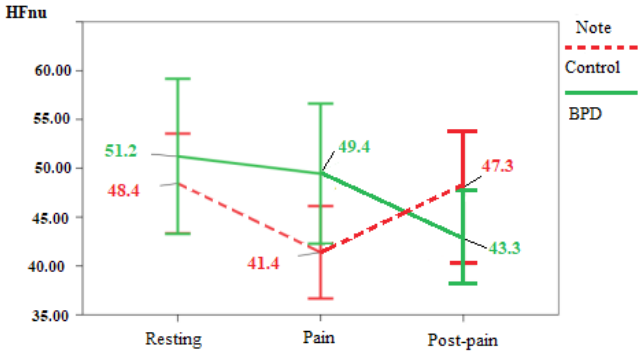

Fig. 5. HFnu values in subjects in control group and BPD group.

The veracity of the comparative differences between the functional tests was more evident within the study groups. In the group with BPD the pain caused a decrease of RMSSD by 10% (44.23 ±10.2 compared to 49.26 ± 10.95 at rest), p <0.05; other changes were not detected (tab. 1).

Table 1

HRV parameters in control and BPD groups

Test / HRV	Study groups					
	Control group (N=69)			BPD group (N=36)		
	Resting	Pain	Post-pain	Resting	Pain	Post-pain
RR (NN) (*ms*)	875.67 ± 18.4	868.84 ± 17.65	878.90 ± 16.80$^{\Delta}$	825.61 ± 24.53	828.05 ± 25.24	830.62 ± 22.71
SDNN (*ms*)	50.23 ± 5.17	49.13 ± 4.14	53.29 ± 4.34$^{\Delta}$	54.51 ± 7.02	50.29 ± 6.45	59.16 ± 6.42 $^{\Delta\Delta\Delta}$
RMSSD (*ms*)	46.18 ± 5.99	42.84 ± 5.06	45.34 ± 5.39	49.26 ± 10.95	44.23 ± 10.2*	50.88 ± 10.1 $^{\Delta\Delta}$
pNN50	16.46 ± 2.76	16.42 ± 2.65	17.84 ± 2.71	16.92 ± 3.36	16.73 ± 3.45	19.41 ± 3.67 $^{\varnothing\ \Delta}$
LF/HF	1.64 ± 0.24	2.10 ± 0.25	1.65 ± 0.27	1.56 ± 0.28	1.63 ± 0.33	2.06 ± 0.35
LFnu	51.35 ± 2.56	58.42 ± 2.37**	52.54 ± 2.38$^{\Delta}$	48.62 ± 3.99	50.30 ± 3.60	56.58 ± 3.38$^{\varnothing\varnothing\Delta}$
HFn	48.44 ± 2.55	41.39 ± 2.36**	47.36 ± 2.38$^{\Delta\Delta}$	51.22 ± 3.97	49.45 ± 3.58	43.28 ± 3.37$^{\varnothing\varnothing\ \Delta}$

Statistical differences between values within study groups. Resting /Pain: *– p<0.05; **–p<0.01; *** p<0.001. Resting/Post-pain: $^{\varnothing}$ – p<0.05; $^{\varnothing\varnothing}$ –p<0.0 $^{\varnothing\varnothing\varnothing}$ p<0.001. Pain/Post-pain: $^{\Delta}$ – p<0.05; $^{\Delta\Delta\Delta}$ –p<0.0$^{\Delta\Delta\Delta}$ p<0.001.

In contrast, after the removal of the painful stimulus, statistically significant differences in several time domain parameters of the HRV are observed in post-pain period versus pain stimulation and resting in the BPD group. pNN50 was 14.7% higher in the post-pain period (19.41 ± 3.67) than at rest (16.92 ± 3.37), p <0.05; SDNN – was 18% higher post-pain (59.16 ± 6.42) compared to (50.29 ± 6.45) during pain, p <0.001; and RMSSD was with 15% higher during post-pain period (50.88 ± 10.1) than during pain stimulation (44.23 ± 10.2), p <0.01 (tab. 1).

The components of the spectral analysis Fourier, LFnu and HFn, were different in post-pain period in comparison with resting and pain test. We reported an increase of LFnu by 16.3% in BPD group in post-pain (56.58 ± 3.39) compared to rest (48.62 ± 3.99), p <0.01, and by 12% in post-pain (56.58 ± 3.38) compared to pain period (50.30 ± 3.60), p <0.05, which indicates an increase in activity pf the sympathetic autonomic nervous system. HFnu was 15.5% lower in post-pain (43.28 ± 3.37) than at rest (51.22 ± 3.97), p <0.01; and by 13% lower in post-pain (43.28 ± 3.37) than in pain period (49.45 ± 3.58), p <0.05.

The time domain parameters: RR (NN), SDNN, RMSSD, pNN50 within the control group did not show significant changes in both pain stimulation and post-pain period compared to rest.

During pain stimulation in the control group, we could see statistically significant differences between the LFnu and HFnu values compared to rest: LFnu was 14% higher during pain (58.42± 2.37) than in resting period (51.35 ± 2.56), p <0.01; HFnu was 15% lower during pain (41.39 ± 2.36) than in resting period (48.44 ± 2.55), p <0.01 (tab. 1).

In the control group the pain stimulation produced statistical changes in LFnu and HFn, and in the BPD group the pain stimulation did not produce statistically valid changes, which speaks about a delayed activation of the autonomic nervous system in subjects with BPD.

During post-pain period in the control group, we observed statistically significant changes in the HRV; the average duration of the RR interval increases by 1% in post-pain period (878.90 ± 16.80) compared to pain stimulation (868.84 ± 17.65), p <0.05; SDNN was increased by 8% in post-pain period (53.29 ± 4.34) in comparison with pain stimulation (49.13 ± 4.14), p <0.05.

HFnu was by 14% higher during post-pain period (47.36 ± 2.38) compared to pain stimulation (41.39 ± 2.36), p <0.01; LFnu was decreased by 10% in post-pain period (52.54 ± 2.38) compared to pain stimulation (58.42 ± 2.37), p <0.05.

In subjects with BPD, higher HFnu values are observed at rest, marking an accentuated vagal modulation of the heart rhythm, and a lower sympathetic influence on the heart rhythm.

During the pain stimulation, a decrease of the vagal activity and an increase of the sympathetic activity on the heart rate were observed in both groups.

In post-pain period, LFnu and HFnu values in subjects with BPD were reversed compared to resting period, which indicates an increase of sympathetic influences on heart rate and a reduction of vagal modulatory effects. The LFnu and HFnu values in these subjects did not return to the initial values in the post-pain period as they did in the control group, but, on the contrary, the accentuation in the dynamics of the sympathetic influence was registered, even compared to the pain period.

Conclusions

1. The results regarding HRV in subjects with BPD, obtained in this study, are in concordance with the results of the studies in the research papers regarding the increased vagal modulation in subjects with BPD at rest, which is reduced during pain stimulation and does not return rapidly to the initial value after removing the painful stimulus, which can be the proof of the inertia of autonomic influences in these subjects.

2. Selecting the subjects for the study with the help of PID-5, translated and adapted for Romanian speakers in the Republic of Moldova, showed that PID-5 is a valid and useful tool for studying HRV in people with personality disorders.

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Authors' ORCID iDs and academic degrees
Svetlana Lozovanu – https://orcid.org/0000-0002-5777-1805, MD, PhD.
Ion Moldovanu – https://orcid.org/0000-0002-1709-0319, MD, PhD.
Victor Vovc – https://orcid.org/ 0000-0001-7624-9644, MD, PhD.
Tudor Besleaga – https://orcid.org/0000-0002-0180-8509, MD, PhD.
Andrei Ganenco – https://orcid.org/0000-0002-9835-5461, MD.

Authors' contributions
IM and VV conceptualized the project and designed the research; TB analyzed and described the data. SL and AG drafted the first manuscript. All authors revised and approved the final version of the manuscript.

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Molecular characterization of the endometrium as a fertility-determining factor

***Mihaela Burac, Natalia Corolcova, Valentin Friptu**

Department of Gynecology, Obstetrics and Human Reproduction
Nicolae Testemitsanu State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova

Authors' ORCID iDs, academic degrees and contributions are available at the end of the article

*Corresponding author: mihaelaburac@gmail.com
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Abstract

Background: Structural transformation of the endometrium during the menstrual cycle is a genetically determined process and is provided by complex molecular-biological interactions aimed at the onset and development of pregnancy. Sex steroid hormones play a key role in endometrial morphogenesis, which mediate or directly affect angiogenesis and immunogenesis.
Conclusions: The primary function of the endometrium is to provide an immuno-privileged site for embryo implantation and to provide a nurturing environment for the fetus during pregnancy. The cyclic differentiation of the endometrium depends on the actions of steroid hormones that act through specific down – stream mechanisms involving complex molecular signaling. The endometrium undergoes repetitive episodes of proliferation, secretion, and menstruation, up to 400 times during a woman's life, without apparent signs of aging. The human endometrium undergoes complex and dynamic changes during the menstrual cycle. Thus, the combination of molecular, endocrine, biochemical, immunological factors leads to a complete transformation of the endometrium during the menstrual cycle. Secretory transformation of the endometrium with an appropriate ratio and distribution of estrogen and progesterone receptor expression, complete angiogenesis and immunological balance determine implantation, placentation and pregnancy development.
Key words: menstrual cycle, endometrium, angiogenesis, immunogenesis.

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Introduction

The endometrium has been an interest for scientists for decades, but its functional activity has yet to be discovered. In the endometrium, complex molecular interactions of biologically active substances take place in order to create optimal conditions for fulfilling its important function, implantation of the embrio and pregnancy development [1, 2, 3]. With the modernization of medicine, subsequently, the knowledge about the structure and functional activity of the endometrium has been detailed and extended. Proliferative and secretory transformation of the endometrium during the menstrual cycle is a genetically determined process based on the balance of the interaction of steroidogenesis, angiogenesis and immunogenesis in the endometrium, starting from the intrauterine development of the fetus [3-6].

Ontogeny of the endometrium

Uterine embryo development begins in the fetus at 8–9 weeks of age. The glandular component of the endometrium comes from the mucosal epithelium of the Muller canals, the cells of the adjacent mesenchyme serve as a source of endometrial stroma and uterine muscle layer. At the beginning of the development, the endometrium is represented by a small cylindrical epithelium, however, as the gestational age advances, the height of the endometrium increases, and from 18 weeks the formation of the first endometrial glands takes place. From 20 weeks of gestation, the active growth of the uterus is observed, which is associated with the development of receptors and the sensitivity of the organ to the mother's sex hormones, especially estrogens. From 24 weeks of pregnancy, the first signs of subnuclear vacuolization are observed in the endometrial epithelium, and the endometrium acquires the characteristics of a secretory tissue. Signs of well-defined secretion in the endometrium and endocervical epithelium are observed from the 28th week with a peak up to 35–36 weeks of gestation, when the placenta secretes estrogen and progesterone maximally [1, 7] .

The endometrium, regardless of age, has a thickness of 0.5 to 1.5 mm, contains a significant number of cells (lymphocytes, fibroblasts, plasmocytes) and a small number of fibers. In the neonatal period, the glandular component of the endometrium is represented by glandular "dives", and only from the first year of life, the glands acquire the characteris-

tics of a typical structure and their number increases. Until puberty, a significant growth and branching of the glands is observed without increasing the thickness of the endometrium [1, 8].

Menstrual cycle in the endometrium

With the onset of puberty, in the endometrium there is a cyclical cascade, complex of molecular and neuroimmunoendocrine interactions under the control of the hypothalamus-pituitary-ovarian axis, which leads to the appearance of the genetically determined menstrual cycle. The endometrium is a complex and interconnected system consisting of glandular epithelium, stroma and vascular component [9].

From the early stage of the proliferation phase to the late stage of the phase of secretion the gland epithelium and stromal cells are characterized by heterogeneity, which provides the processes of cell transformation. With the beginning of the proliferation phase, endometrial reepithelization begins from the process of migration of epithelial cells from the growing glands to the beginning of proliferative activity of stromal and epithelial cells. This process fully covers the entire wound surface of the uterus, and there is a rapid restoration of the functional layer [9-12].

The use of scanning electron microscopy of the menstrual endometrium showed that epithelial cells arise from stromal mesenchymal cells in desquamated areas, and not only from epithelial glands, which suggests reprogramming of endometrial stromal cells even in the phase of menstrual decay [13, 14]. In this case, mesenchymal cells change their characteristics and become epithelial cells, this process is known as the mesenchymal-epithelial transition (MET). Evidence for this hypothesis was obtained in an experiment in mice using the cytoskeleton protein pancytokeratin and the vimentin stromal cell marker. Significant changes in MET were detected in endometrial cells 24 hours after progesterone withdrawal [15, 16]. In was demonstrated the activation of proliferation processes in areas of damaged endometrial stroma under the influence of cytokeratin and osteopontin, similar to the MET process [16]. Therefore, it can be assumed that the basal layer of the endometrium promotes reepithelization of the desquamated surface.

There is a reverse MET process – the epithelial-mesenchymal transition (EMT), which is necessary for wound healing and the development of fibrosis [17]. The role of EMT in the endometrium remains unclear, but it is likely that the balance of EMT and MET is of great importance for the processes of full repair of the endometrium in the desquamation phase. Strict control of these factors in the endometrium enables the tissue to heal without scarring [18].

Vascular remodeling and angiogenesis

Within the myometrium, the arcuate arteries arise from the uterine and ovarian arteries, which in turn give rise to radial arteries. After crossing the endometrial – myometrial junction, they branch to form the basal (anastomosing) and spiral (terminal) arteries. The former supply the basal layer and the latter the functional layer of the endometrium. Branching of the spiral arteries occurs throughout the functional layer. Just below the surface they break up into a prominent subepithelial plexus, which drains into venous sinuses. Each spiral arteriole supplies tissue with an approximate endometrial surface area of 4 – 9 mm [19, 20].

Unlike other vascular beds, the endometrial vasculature undergoes cycles of growth and regression during the menstrual cycle [21]. The proliferative phase growth in endometrial thickness is accompanied by growth of the vascular tree [22]. By the middle of the late proliferative phase the sprouting terminal branches of the spiral arteries become somewhat coiled. By the middle of the secretory phase the spiral arteries ascend from the basal to the functional layer [20, 22].

There are two main mechanisms for the formation of new blood vessels: vasculogenesis, *de novo* development of vessels and angiogenesis, the creation of new microvessels from pre-existing vessels. Angiogenesis may occur by sprouting/branching or elongation, in addition, circulating endothelial cell progenitors may be incorporated into existing vasculature to contribute to these processes. For perfusion of growing tissue, adequate angiogenesis is required. Angiogenesis is thought to occur in three phases of the menstrual cycle: during menses, when vascular repair is occurring, during the proliferative phase, coinciding with the estrogen-driven rapid tissue growth, and during the secretory phase, associated with the elaboration of the spiral arterioles. Angiogenesis normally involves endothelial cell activation, degradation and breakdown of the basal lamina, migration and proliferation of the endothelial cells, fusion of sprouts, and tube formation. By the 5–6th day of the menstrual cycle, estradiol synthesis is increasing by growing follicles, which directly stimulates endometrial neovascularization by expression of angiopoietin-2 (Ang-2) in the endothelium. Estrogen does not significantly affect endometrial repair in the early stage of the proliferation phase. However, during the middle and late stages of the proliferation phase, when the main mechanism of angiogenesis is an increase in vessel length, estrogen, together with VEGF (vascular endothelial growth factor), synthesized by stromal cells, provides estrogen-dependent regeneration and increased vascular permeability [21, 23, 24]. In an experiment on animals undergoing ovariectomy, three peaks of the effect of VEGF on the endometrium were shown: in the early stage of the proliferation phase on the surface epithelium, in the middle stage of the proliferation phase on stromal fibroblasts and during the late stage of the secretion phase on the glandular component [23]. The significance of the vascular component in endometrial regeneration has been confirmed by studies of stromal growth factor (SDF-1) via pro-fibrotic CXCR4 or pro-regenerative CXCR7 receptors. Stromal growth factor (SDF-1) is present in all phases of the menstrual cycle, and CXCR4 expression is expressed in the early proliferative phase in both epithelial and endothelial cells [25].

The physiologic consequences of angiogenesis are reflected in changes in endometrial blood flow. By measuring the clearance of radioactive xenon gas, highest endometrial perfusion was reported between days 10 and 12 and days 21

and 26 of the cycle. Microvascular perfusion has been assessed by laser Doppler flowmetry with transvaginal placement of a fiberoptic probe into the uterine cavity. With use of this technique, endometrial perfusion was found to be highest during the proliferative phase and the early secretory phase, not too dissimilar from the finding based on xenon clearance. Uterine blood flow is greatest in the fundus, and higher flow rates are associated with better outcomes in assisted reproduction. Notably, diminished uterine blood flow has not been found in the perimenstrual period, but these methods cannot easily identify localized areas of vasoconstriction [26].

Immunology of the endometrium

The uterus is an immunologically privileged organ: it can accommodate tissue invasion by immunologically semiforeign placental cells, yet maintain mucosal immune defenses against ascending foreign organisms, and provide a system to efficiently clear the endometrial detritus that results from menstruation. Remarkably, the endometrium also uses mechanisms of acute inflammation during normal, hormonally regulated physiologic processes, including menstruation and embryo implantation. These acute inflammatory episodes are quickly resolved, avoiding the consequence of scarring and dysfunction. Despite the description of critical active processes to resolve inflammation in other tissues and the clear relevance to endometrial physiology and pathophysiology, mechanisms that resolve endometrial remain largely unstudied [27-29].

The complex requirements of uterine immunity and tolerance use overlapping and redundant mechanisms dependent on both innate and adaptive branches of the immune system. The onset and development of pregnancy is inextricably linked with the presence of physiological and pathological inflammatory-immune reactions in the endometrium and directly in the nidation zone [30-32]. One of the important features of a woman's reproductive tract is the constancy of the physiological microbial population and the prevention of inflammatory reactions [33]. Endometrial immune processes, as with other uterine functions, but unlike those for other mucosal immune sites, are markedly influenced by cyclic and pregnancy-specific changes in sex steroid concentrations and possibly by human chorionic gonadotropin [34, 35].

The endometrium is populated by bone-marrow derived immune cells, as well as endometrial epithelial and stromal cells that demonstrate immune functions [36]. As is the case for many epithelial cells, endometrial epithelium express members of the Toll-like receptor family (TLR2 to 6, 9, and 10), which detect pathogen products and trigger a cellular response to these "foreign" molecules, including peptidoglycans from Gram positive bacteria (TLR2), lipopolysaccharide from Gram negative bacteria (TLR4), and unmethylated CpG islands found in bacterial DNA (TLR9) [33]. The endometrium also produces host defense molecules, defensins, as well as cytokines and chemokines. Uterine lymphoid and myeloid cells play roles in tissue defense, immune modulation, angiogenesis, and tissue remodeling [37-39]. These cells are present in the fallopian tubes, uterus, and cervix, with the fallopian tubes and uterus containing a higher proportion of leukocytes than the cervix and vagina [40].

The endometrial innate and adaptive immune systems are regulated by steroid hormones. For example, progesterone induces a local Th2-type cytokine response in the uterus, which includes an increase in IL-4, IL-5, and IL-15 and downregulation of the IL-13 receptor α2, which is a negative regulator of the anti-inflammatory cytokine, IL-13, and powerful inhibitor of the Th2 response [35, 41, 42]. The Th2 response is believed to counter proinflammatory processes in the endometrium that could lead to rejection of the embryo. Steroid hormone-directed alterations in endometrial chemokine production influence the trafficking of blood leukocytes in the reproductive tract. Further, actions of progesterone are important in the overall immune suppressive phenotype adopted by the receptive endometrium [33, 43].

During the secretory phase, there is a profound recruitment of leukocytes into the endometrium starting in the perivascular locations around spiral arterioles and glandular epithelium [44]. The progesterone-induced alteration in endometrial cytokine/ chemokine production contributes to this recruitment [40]. Cytokines IL-1, IL-11, IL-15, LIF, and TGF-β regulate trafficking of leukocytes to the endometrium [44, 45]. IL-15 recruits NK cells into the endometrium, and IL-15 knockout mice lack NK cells. Locally acting prostaglandins (PG), including PGE along with VEGF, modulate vascular permeability [46-49]. Cyclooxygenase-2 (COX-2), a rate limiting enzyme that regulates the biosynthesis of PGE_2, is critical to implantation in the mouse, and in animal models, PG are required for initiation and maintenance of decidualization. Blockade of COX-2 prevents decidualization in mice and clearly plays a role in endometrial function surrounding pregnancy, with reduced COX-2 associated with implantation failure [50-52].

The immune component of the mucous membrane of the female genital tract in different parts of the genital tract is represented by the predominant population of T cells, macrophages / dendritic cells, natural killer cells (NK), neutrophils and mast cells [33, 37]. Macrophages (CD68), plasmocytes (syndicans) and B cells are present in the endometrium at all stages of the menstrual cycle in small quantities. Also during the proliferative phase, syndicans induce angiogenesis [53, 54]. The basal layer of the endometrium contains true lymphoid follicles formed from germinal centers, the bright centers of which consist of B cells surrounded by T cells and an external halo of macrophages (CD14). In the late stage of the proliferation phase and in the phase of secretion, lymphoid follicles increase in size, with B cells expressing CD19 and T cells expressing almost exclusively CD8 and extremely rare CD4 [44].

In the functional layer of the endometrium of the proliferation phase, there are predominantly cytotoxic T-lymphocytes (CD8 +), which have increased cytolytic activity compared to the secretory phase of the cycle. Moreover, the suppression of the cytolytic activity of CD8 + is noted only in the secretory endometrium and fallopian tubes, in contrast to the cervix. The content of the number of cytotoxic

T-lymphocytes (CD8 +) and T-helpers (CD4 +) in the normal endometrium is up to 10 cells in the field of view, B-lymphocytes (CD20 +) up to 3 cells in the field of view [44]. An increase in the number of cells of cytotoxic T-lymphocytes, B-lymphocytes and the presence of plasmocytes (CD138 +) indicates the presence of chronic endometritis [46]. The process of decidualization of the endometrial stroma is characterized by a limiting effect on inflammatory processes in the functional layer, while the basal layer remains intact, which is crucial for effective reparative processes of the endometrium. In addition, progesterone blocks the activation of metalloproteinases (MMP) during the secretory phase of the cycle [55, 56].

The immunological cell composition of the endometrial secretory phase is represented by NK cells that express surface receptors CD56 +, CD16 +, CD3 + and are phenotypically different from peripheral blood NK cells. An increase in CD56 + during the middle stage of the secretion phase with predominantly periglandular and perivascular localization is associated with maintaining the immune tolerance of the mother's body to the onset and developing pregnancy [44, 56]. By the end of the secretion phase in the endometrium, the population of neutrophilic leukocytes increases significantly (up to 7–15%), which contain high levels of MMP for initiating endometrial decay. White blood cells do not have estrogen and progesterone receptors and penetrate the endometrium by chemotaxis in response to physiological and pathological inflammatory reactions in the tissue [44]. A feature of neutrophils in this period is resistance to apoptosis and hypoxia under the influence of inflammatory mediators, which enhances tissue damage [45, 46].

Progesterone, in addition to secretory transformations of the endometrium, also affects the contractility of the myometrium. A decrease in progesterone receptors expression in the late stage of the secretion phase leads to activation of the myometrium and an increase in contractile activity in the menstrual phase, while the level of progesterone in the blood serum does not correlate with the concentration of progesterone in the myometrium [12, 27, 28]. In the desquamation phase, an excessive or prolonged inflammatory response can lead to significant tissue damage and polymenorrhea, while the level of tumor necrosis factor and pro-inflammatory cytokines increases, and expression of cyclooxygenase-2 (COX-2) mRNA also increases [31]. 36 hours after the onset of menstruation in the endometrium, reparative processes begin. Thus, the combination of molecular, endocrine, biochemical, immunological factors leads to a complete transformation of the endometrium during the menstrual cycle.

An immunotolerance maternal immune response is essential for the acquisition of endometrial receptivity and the success of pregnancy [57]. Factors that support a more suppressed immune environment, including the recruitment of T regulatory cells (Tregs) and a shunting away from a pro-inflammatory, Th1/Th17 responses are central to our understanding of infertility and pregnancy loss associated with various inflammatory conditions.

Conclusions

The human endometrium undergoes complex and dynamic changes during the menstrual cycle. Thus, the combination of molecular, endocrine, biochemical, immunological factors leads to a complete transformation of the endometrium during the menstrual cycle. Secretory transformation of the endometrium with an appropriate ratio and distribution of estrogen and progesterone receptor expression, complete angiogenesis and immunological balance determine implantation, placentation and pregnancy development.

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Authors' ORCID iDs and academic degrees
Mihaela Burac – https://orcid.org/0000-0002-1672-9467, MD.
Natalia Corolcova – https://orcid.org/0000-0002-5155-2804, MD, PhD.
Valentin Friptu – https://orcid.org/0000-0003-2804-899X, MD, PhD.

Authors' contributions
MB designed the trial and drafted the first manuscript. NC interpreted the data. VF revised the manuscript critically. All the authors revised and approved the final version of the manuscript.

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Transcatheter aortic valve implantation – new era in treatment of aortic stenosis

Oleg Calenici

Department of Normandy, Hospital Centre, Lillebonne, France

Authors' ORCID iD, academic degrees and contributions are available at the end of the article

Corresponding author: dr.oleg.calenici@gmail.com

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Abstract

Background: The presence of aortic narrowing is common in the elderly and the prognosis is very poor in symptomatic patients. Prior to the era of percutaneous treatment of aortic stenosis, surgical aortic valve replacement (SAVR) was considered the "gold standard" of symptomatic aortic stenosis treatment. However, the records have shown that about 40% of patients at that time were not operated due to their age and their cardiac and non-cardiac comorbidities. The subsequent work was the implantation of aortic valve by transfemoral approach – transcatheter aortic valve implantation (TAVI). Several studies have acknowledged the place of aortic valvuloplasty which has become the reference technique for patients with contraindications or high surgical risk and even recently appeared as at least equivalent to, or even superior to, the surgery in patients at intermediate risk. This development was accompanied by a renewed interest in aortic valvuloplasty, a TAVI that cannot reasonably be executed at first attempt in some patients. In Europe, where this technique was born, the great experience that has been gained has led to a gradual simplification of the procedure. The purpose of this article is to describe the state of the art of TAVI and to discuss its future.

Conclusions: The aortic stenosis disease affects a large scale of people across the globe. The appearance of a new treatment method TAVI opens new era of treatment of this disease. The new TAVI method is a less invasive procedure than an open heart surgery and can be used in almost all the cases of patients with an aortic stenosis.

Key words: aortic stenosis, transcatheter aortic valve implantation, surgical aortic valve replacement.

Cite this article

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Introduction

Calcified aortic stenosis is the most severe form of aortic valve disease and it is characterized by fibrocalcic remodeling. This remodeling process begins with a deposition of lipoproteins and chronic inflammation, resulting in osteogenic differentiation of valvular interstitial cells and active calcification of the layers [1, 2]. Despite similarities with the atherosclerotic process, no pharmacological treatment slowed the progression of the aortic stenosis. Large-scale epidemiological studies have shown an annual incidence of calcific aortic stenosis in the range of 0.36 and 0.37 per 1000 hospital-treated patients [3, 4]. In the general population, the incidence is higher (4.9 per 1000) when calculated on the basis of systematic echocardiographic examination. The prevalence of calcific aortic stenosis is estimated at 0.4% in the general population and between 1.3 and 1.7% in patients over 65 in developed countries [5-7]. The prevalence of calcified aortic stenosis increases significantly after age of 65 and it reaches its severe form of 3.4% after the age of 75, with 75% of symptomatic patients [8]. The natural course of severe symptomatic calcific aortic stenosis is particularly dark, as shown by the 5-year mortality rate of 60% after the initial hospitalization. Mortality is increased in cases of heart failure or in octogenarians with comorbidities [9, 10]. The reliability of the epidemiological data of valvulopathy has important implications for the planning of therapeutic resources. The number of patients with aortic stenosis is expected to triple in the next 50 years [11-13].

History of transcatheter aortic valve implantation (TAVI)

The development of TAVI has been a long odyssey since the birth of the concept in the early 90s. The history began in 1985 in Rouen with the introduction of the aortic dilatation balloon by Alain Criber. After considerable international interest in this technique, its limitations, particularly early valvular restenosis, led to the development of the "percutaneous aortic valve" concept. Faced with the absence of any industrial support, a start-up, "Percutaneous Valve Technologies", was created which allowed the development and testing on animals of the first balloon-expandable dentures in 2000, before the first human implantation took place at the University Hospital Center on 16 April 2002 in Rouen [14]. The 2004 acquisition of this start-up by Edwards Lifesciences was the starting point for significant technological improvements and increasing interest in TAVI, while two years later a competing self-expandable prosthesis was introduced the Medtronic Core Valve. Multiple controlled registers and studies with these two prostheses have resulted in the extraordinary expansion of the TAVI that we know today, with inclusion in the European and American recommendations since 2012 and a gradual and recently validated expansion of indications to patients with less risk [15]. Pre-

TAVI Patient Assessment and selection is a key step in the implementation of a TAVI and should involve a multidisciplinary team of cardiologists, imaging specialists, cardiac surgeons and geriatricians if needed or other specialists [16-21]. The investigations prior to TAVI should include: Echocardiography to confirm the severity of aortic stenosis, analyze aortic valve and ascending aortic morphology, size and function of the left ventricle to rule out dynamic ventricular obstruction and evaluate the mitral valve; coronary angiography to determine revascularization options. The multi-cut coronary computer tomography (CT) is essential to the therapeutic decision, especially for the selection of the best approach in terms of vessel size, tortuosity and calcification. When the indication of a TAVI is retained, a scan of the arteries is performed from the ascending thoracic aorta to the arteries of the lower limbs [22].

For the femoral approach, the ratio of the outer diameter of the vest to the minimum diameter of the vessel should be less than 1.1 in the absence of calcification. When the femoral approach is not feasible, the scanner can make it possible to evaluate the feasibility of another pathway (transcarotide, transaortic, subclavian or transapical).

Transfemoral approach after the initial approach combining a surgical and percutaneous approach is the approach currently entirely percutaneous. This is often the preferred approach because it offers the largest arterial diameters and has a lower rate of complications.

Left subclavian / transaxillary approach: it allows TAVI with the CoreValve prosthesis in patients who cannot benefit from a femoral approach, without requiring thoracotomy. Nevertheless, surgical exposure is required, with a procedure frequently performed under general anesthesia.

Transapical approach: it allows a direct anterograde access via the tip of the VG, without passing through the aorta. This is a surgical approach that requires a left anterolateral intercostal incision, under general anesthesia. It is associated with higher mortality compared to the transfemoral route.

Transaortic approach: this approach consists of a ministernotomy at the level of the ascending aorta, under general anesthesia. The aortic puncture site and the thoracic wall are surgically sutured at the end of the procedure. Other approaches have been developed more recently, such as the transcarotid approach or the brachiocephalic arterial trunk (fig. 1) [23-26].

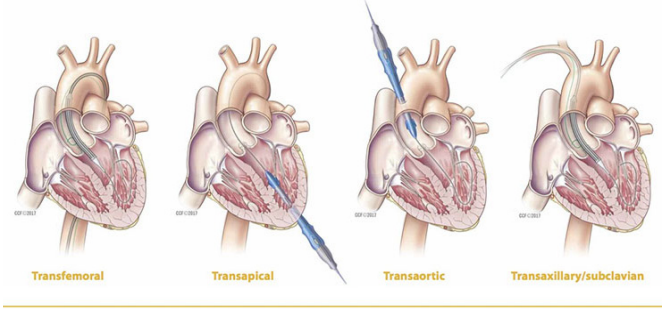


Fig 1. Different approach of TAVI.

In addition, in terms of calibration and selection of the appropriate valve type, CT has become the method of choice for assessing horizontal and ascending aorta, Valsalva sinus size, diameter and shape of the aortic annulus, the volume of calcifications, the bi- or tricuspid nature of the valve, the distance between the valvular ring and the ostium of the coronaries, the presence of a septal margin and possible calcifications in the flush chamber. These explorations make it possible to choose the most appropriate calibers and types of valve and the best incidence for the positioning of the prosthesis. For patients with coronary disease and severe aortic stenosis, the strategy is most likely to allow complete vascularization.

Patients treated with TAVI should be evaluated on a case-by-case basis by the multidisciplinary team, in the extent and complexity of the coronary lesions, the risk of myocardial infarct (risk of myocardial ischemia) and the potential complexity of the angioplasty, as well as the presence of any comorbidities. It should be emphasized that the recently published European Society of Cardiology (ESC) recommendations on myocardial revascularization advocate angioplasty in patients with TAVI-treated coronary artery disease who have a stenosis greater than 70% in the proximal coronary segments [27-29].

The angioplasty and TAVI performed separately, or the two procedures performed concomitantly are strategies considered acceptable with respective advantages and disadvantages that must be carefully considered on a case-by-case basis.

Technique of prosthesis implantation

Long considered to be at high risk of complications, percutaneous aortic valvuloplasty has become a relatively simple act of interventional cardiology, well tolerated, requiring only local anesthesia and short hospitalization.

When the transfemoral route is chosen, a "crossover" technique (involving a wire placed in the contralateral artery to allow delivery of a balloon or stent to treat the access vessel in case of injury) is usually performed to "protect" the artery in case of vascular complication [30].

A temporary pacing wire (TPW) is positioned in the right ventricle via the jugular or femoral vein and may be required during balloon aortic valvuloplasty (BAV), implantation of the TAVI prosthesis, post-dilatation or if the patient develops significant conduction disturbance following valve deployment.

The aortic valve is usually crossed with the aid of a Judkins right 4 (JR4) or Amplatz left 1 (AL1) diagnostic catheter and a soft straight-tipped wire. This is then exchanged for a stiffer wire taking care to ensure that this is free of the mitral valve apparatus. Originally, wires (e.g., Amplatz Super Stiff™, Boston Scientific, Marlborough, MA, USA) were manually shaped to create a curve at the end to reduce the risk of ventricular injury during valve deployment. However, dedicated pre-shaped wires (e.g., the Safari™ pre-shaped TAVI guidewire; Boston Scientific)) have more recently been developed that have better memory (and therefore maintain their shape) to further reduce the risk of ventricular injury

(fig. 2). Stiffer wires, including the Lunderquist® Extra Stiff wire (Cook Medical, Bloomington, IN, USA) or the Back-up Meier™ guidewire (Boston Scientific), may be used when greater support is required to deliver the TAVI device (e.g., in the setting of severe aortic tortuosity).

The aortic valve is initially crossed with a soft-tipped straight wire with the aid of an Amplatz left 1 catheter (fig. 2 A). Once the valve is crossed (fig 2 B), the wire is exchanged for a stiff wire with a curved tip (white arrow) to minimise ventricular injury (fig. 2 C) over which the TAVI device is then advanced.

Transcatheter valves are positioned prior to deployment with the aid of aortography, fluoroscopy and, in some instances, transoesophageal echocardiographic guidance. Balloon expandable valves require rapid ventricular pacing (180-220 beats per minute) for deployment to reduce cardiac output and avoid inaccurate valve implantation [31]. Other devices may not routinely require ventricular pacing, although this may still be useful in instances when valve positioning is challenging (e.g., horizontal aorta).

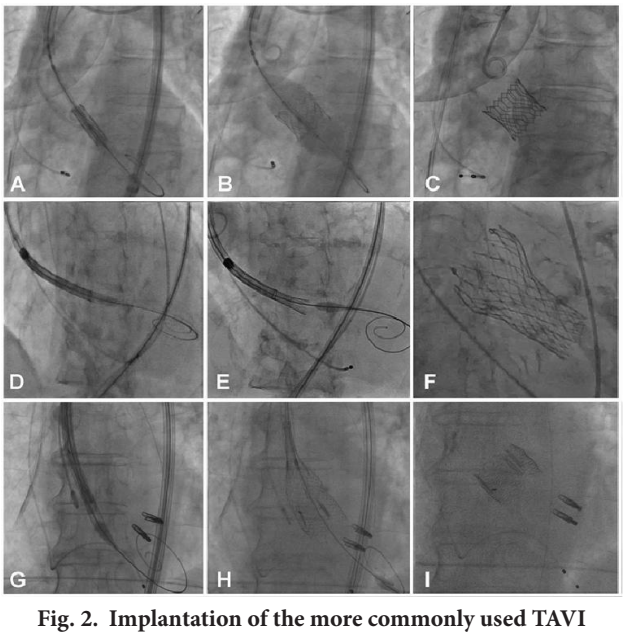


Fig. 2. Implantation of the more commonly used TAVI prostheses.
Initial position of the balloon-expandable Edwards SAPIEN 3 valve (A), deployment (B) and final appearance (C). Initial position of the self-expanding Medtronic Evolut R valve (D), deployment (E) and final appearance (F). Appearance of the mechanically deployed Boston Scientific Lotus valve (G), deployment (H) and final appearance (I).

The goal is to achieve a reduction of the transvalvular aortic gradient of approximately 50% (if possible, less than 25 mm Hg) and an increase of the aortic surface of 100% (example: 0.5 to 1 cm^2).

If the result is insufficient, a larger diameter balloon catheter is used. At the end of the procedure, arterial vascular hemostasis is provided by a percutaneous Angio-Seal 8-Fr (Terumo) or Proglide (Abbott) arterial closure system. The patient can usually leave the hospital the day after the procedure [32].

Current indications of TAVI

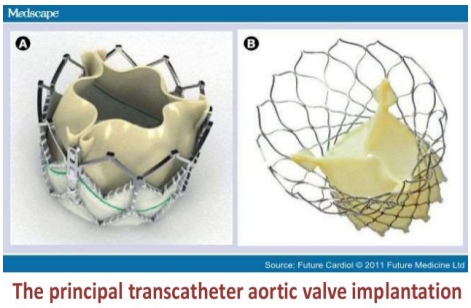
Aortic valve replacement surgery has been the standard treatment for many patients with aortic stenosis for many years and provides symptomatic relief and increased survival. Over the past 15 years, more than 400.000 percutaneous aortic valve implantations have been performed in more than 75 countries. The TAVI technique has now reached maturity, the intervention being standardized and the results predictable.

In high risk patients

According to European (2012) and North American (2014) recommendations, TAVI is currently indicated in inoperable patients or is considered an alternative to surgery in high-risk patients [6, 33-36].

These indications are based on the results of the PARTNER 1 studies with balloon deployed valves (Sapien, Edwards Lifesciences) and the US CoreValve study with self-expanding valves (CoreValve, Medtronic). The PARTNER 1B study, published in 2010, randomized 358 patients, with tight RA and considered inoperable, between a transfemoral TAVI with the 1st generation Sapien valve and medical treatment. The primary outcome (death) at 1 year was 30.7% in the TAVI group and 50.7% in the medical group ($p < 0.001$). The PARTNER 1A study, published in 2011, is a randomized non-inferiority study comparing in 699 patients with tight RA and considered to be at high risk for TAVI transfemoral (2/3 of patients) or transapical with valve 1st generation Sapien and AVR. The primary outcome (death) at 1 year was 24.2% in the TAVI group and 26.8% in the AVR group ($p = 0.62$). Therefore, the PARTNER study had indeed shown that in nonoperable patients with severe aortic stenosis, a TAVI significantly reduced mortality at one year compared to drug treatment3 and that in patients at very high risk of surgery. TAVI and conventional surgical replacement had a mortality rate comparable to one year [37, 38].

In 2014, another study conducted with the 1st generation CoreValve valve was also published. This study compared TAVI and surgical therapy in 795 patients with aortic stenosis and considered at high surgical risk. The primary outcome (death) at 1 year was 14.2% in the TAVI group and 19.1% in the AVR group ($p = 0.04$) [39] (fig. 3).



The principal transcatheter aortic valve implantation devices currently in use:
(A) Edwards Sapien XT bioprosthesis and
(B) Medtronic CoreValve® bioprosthesis

Fig. 3. The TAVI devices.

The results of these pivotal studies have therefore allowed the entry of TAVI into the European recommendations for the management of patients with aortic stenosis (2012). TAVI is limited to inoperable patients and is considered an alternative to surgery in high-risk patients. Patients must have a life expectancy greater than 1 year, procedures must be validated by a medical-surgical meeting (Heart Team) and performed in experienced centers with on-site cardiac surgery [40-42].

Scores to predict surgical risk

The operative risk of aortic valve replacement (AVR) in a patient with severe aortic stenosis is usually assessed by risk scores.

In Europe, the most used score is the recently updated EuroSCORE (European System for Cardiac Operative Risk Evaluation) in the form of EuroSCORE2, while in the United States, the most commonly used score is STS (Society of Thoracic Surgeons score). The validity of these risk scores is usually assessed by their calibration (ratio of observed mortality to expected mortality) and their performance (area under the ROC curve). The first-generation logistics EuroSCORE is poorly calibrated (because it overestimates mortality) and the area under the ROC curve (0.62) is imperfect to discriminate at-risk patients. On the other hand, the EuroSCORE 2 and the STS are better calibrated (ratio between the observed mortality and the expected mortality close to 1) and more discriminating (area under the ROC curve between 0.73 and 0.75) [43-48].

EuroSCORE 2 and STS are scores to assess the risk of cardiac surgery before surgery, and the expected mortality in this type of patient. The higher the score, the higher the risk.

Patients whose EuroSCORE2 or STS score is >8% are considered at high risk and those for whom these scores are <4%, are at low risk.

Patients are usually considered to be at intermediate risk when the EuroSCORE2 or STS score is between 4 and 8% (This means that the risk of death expected at 30 days in case of RVA is between 4 and 8%).

These risk scores have many limitations because they do not take into account a certain number of cardiac or extracardiac co-morbidities that will also impact on the operative risk. Patients with a porcelain aorta (massive circumferential calcification of the ascending aorta), regardless of the level of risk, are usually excluded from surgery because it is impossible to perform aortic clamping during the procedure.

In addition, patients with a hostile chest due to deformities or a history of radiotherapy are also usually considered by surgeons as high-risk patients. Some extracardiac comorbidities, such as advanced hepatic cirrhosis and severe chronic respiratory insufficiency are also not taken into account. Finally, the fragility, autonomy, and cognitive functions of the patient, well known to geriatricians, are not taken into account by these risk scores even though it has been well demonstrated that they have a major impact on morbidity-operative mortality.

Outcomes in intermediate risk patients

Interestingly, the PARTNER 2 study included patients with a low STS score (5.8%) thus presenting a surgical risk this time intermediate. In this study, patients with severe aortic stenosis had comparable mortality or stroke rates after two years, regardless of technique.

However, comparing only those patients who received TAVI transfemoral to surgical patients, the two-year mortality was significantly lower in the TAVI arm (p = 0.05). These results were confirmed in 2017 by the SURTAVI study, which also included patients at intermediate risk (STS score 4.5%) and in which TAVI and conventional surgery were equal to two years in terms of mortality and stroke [49-51].

Three major studies have been reported in patients with tight RA and lower surgical risk. The first study (NOTION), conducted in Denmark and Sweden, involved a reduced population of 280 patients (70 years old), regardless of the level of surgical risk. Patients were randomized for TAVI with a CoreValve valve or surgery. The primary endpoint at 1 year (associating death, heart attack and stroke) was similar in both groups (13.1% vs. 16.3%; p - 0.43).

The second study was conducted with the 2nd generation Sapien valve (model XT, PARTNER 2) and was published in 2016. This study randomized 2032 patients, considered intermediate risk, between TAVI and conventional surgery. The primary endpoint for 2-year judgment (combining death and stroke) was similar in the two groups (19.3% vs. 23.1%; p - 0.25) in the whole group studied. In contrast, the occurrence of death or stroke was significantly lower in the transfemoral TAVI group compared to surgery (16.8% vs. 20.4%; p - 0.05).

The latest study involves the 3rd generation Sapien valve (Sapien 3). This valve has the peculiarity of having an external shell in its ring portion to greatly reduce the incidence of paravalvular leaks. In addition, the size of the catheter allowing implantation has been significantly reduced to 14-16 F, reducing vascular complications. This is a registry comparing 1.077 intermediate-risk patients treated with TAVI and 944 patients treated with RVA paired with the surgical cohort of the PARTNER 2 study. The primary endpoint (combining death, stroke) at 1 year was significantly lower in patients treated with TAVI (10.8% vs. 18.8%; p - 0.001) [52].

Thus, between 2012 and 2017, large randomized studies have demonstrated the value of TAVI in intermediate-risk patients, so it is logical that the number of TAVIs doubled between 2012 and 2015, essentially, since the reimbursement of TAVI by Social Security [53] (fig. 4).

Taking these recent studies into account, the new recommendations of the European Society of Cardiology, published in 2017, have evolved quite a bit in favor of TAVI. These are patients with a risk score (EuroSCORE 2 or STS score) of between 4 and 8%. These new recommendations include promoting the TAVI approach to any intermediate-risk patient 75 years of age or older.

Finally, various studies are currently looking at TAVI in low-risk patients. The NOTION study (280 low-risk pa-

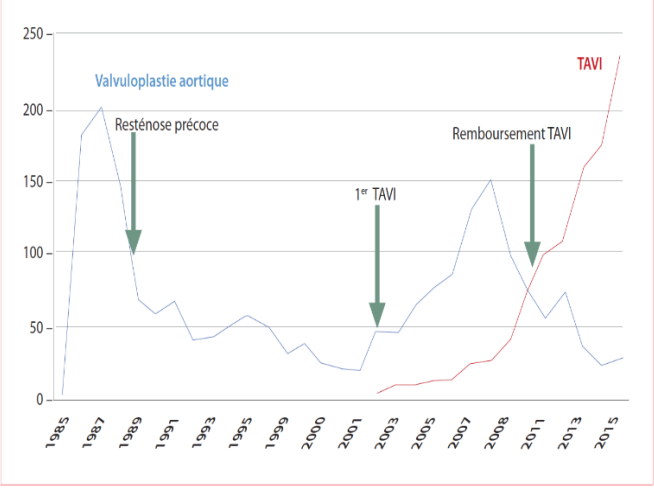


Fig. 4. The decrease in the number of surgical aortic valvuloplasties performed at the University Hospital in Rouen, France, since the TAVI reimbursement in 2010.

tients), whose results at five years have just been presented, thus observed a similar mortality between the two strategies. New data specific to patients at low risk of surgery is therefore to be expected soon [54-56].

For low risk patients (EuroSCORE 2 or STS score – 4%), but with other risk factors for conventional surgery such as significant fragility, aorta "porcelain" or radiation sequelae, different criteria should be taken into account when choosing between conventional surgery or TAVI during a Heart Team were listed (tab. 1).

In addition, it is important to know the theoretical life expectancy of patients according to their age before making a choice between a TAVI and an SAVR (tab. 1).

Evolution of anesthesia

Historically, local anesthesia with conscious sedation had been used during the very first TAVI in 2002 but, at present, practices depend mainly on the experience and habits of the medical hospitals.

General anaesthesia (GA) is required for surgical access sites (e.g., transapical or transaortic). However, improvements in pre-procedural assessment (particularly using advanced imaging of the aorta and peripheral vasculature using computed tomography and echocardiography) and engineering advances in prosthesis delivery systems (e.g. smaller delivery sheaths) have enabled the increasing use of conscious sedation and local/regional anaesthesia. Whilst no randomized studies have been conducted to ascertain if these are superior to GA, the advantages include shorter procedure times, the elimination of risks associated with GA and faster patient recovery [57, 58]. However, due to the better profile of the equipment and the ever-increasing experience of the different teams, local anesthesia is increasingly used during the TAVI and convincing data support feasibility and safety. In terms of differences in prognosis between these two approaches, the data is still unclear. Systematic reviews of the literature and meta-analyses did not observe differences in mortality or stroke incidents, but two more recent studies, based on records of 1737 and 16543 patients, suggest that local anesthesia with conscious sedation may be associated with lower hospital mortality [59].

Table 1

Aspects to be considered by the Heart Team for the decision between SAVR and TAVI in patients at increased surgical risk

	Favours TAVI	Favours SAVR
Clinical characteristics		
STS/EuroSCORE II <4% (logistic EuroSCORE I <10%)[a]		+
STS/EuroSCORE II ≥4% (logistic EuroSCORE I ≥10%)[a]	+	
Presence of severe comorbidity (not adequately reflected by scores)	+	
Age <75 years		+
Age ≥75 years	+	
Previous cardiac surgery	+	
Frailty[b]	+	
Restricted mobility and conditions that may affect the rehabilitation process after the procedure	+	
Suspicion of endocarditis		+
Anatomical and technical aspects		
Favourable access for transfemoral TAVI	+	
Unfavourable access (any) for TAVI		+
Sequelae of chest radiation	+	
Porcelain aorta	+	
Presence of intact coronary bypass grafts at risk when sternotomy is performed	+	
Expected patient–prosthesis mismatch	+	
Severe chest deformation or scoliosis	+	
Short distance between coronary ostia and aortic valve annulus		+
Size of aortic valve annulus out of range for TAVI		+
Aortic root morphology unfavourable for TAVI		+
Valve morphology (bicuspid, degree of calcification, calcification pattern) unfavourable for TAVI		+
Presence of thrombi in aorta or LV		+
Cardiac conditions in addition to aortic stenosis that require consideration for concomitant intervention		
Severe CAD requiring revascularization by CABG		+
Severe primary mitral valve disease, which could be treated surgically		+
Severe tricuspid valve disease		+
Aneurysm of the ascending aorta		+
Septal hypertrophy requiring myectomy		+

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CABG – coronary artery bypass grafting; CAD – coronary artery disease; EuroSCORE – European System for Cardiac Operative Risk Evaluation; LV – left ventricle; SAVR – surgical aortic valve replacement; STS – Society of Thoracic Surgeons; TAVI – transcatheter aortic valve implantation.

TAVI for the treatment of aortic bioprosthesis degenerations

The new 2017 recommendations also enthrone TAVI as a therapeutic modality for the treatment of aortic bioprosthesis degeneration. Aortic replacement with bioprosthesis, about one in two patients will have degeneration with a necessary surgical reintervention rate in 10-30% of cases. As patients with aortic bioprosthesis degeneration are generally frail, elderly and that, by definition, they have a history of heart surgery, a percutaneous approach by a TAVI called valve-in-valve is at first sight attractive. Since the first case described in 2007, this procedure has been increasingly used in inoperable or very high-risk surgical patients with encouraging results. In the largest international registry of patients with aortic bioprosthesis degeneration at very high surgical risk, the one-year survival rate after a TAVI valve-in-valve is indeed greater than 80%. This new indication to achieve a TAVI is now recognized in the guidelines of the European Society of Cardiology for inoperable or considered to be at high risk of surgical re-intervention. However, because the TAVI valve-in-valve situation is more complex than the management of a conventional aortic stenosis, it needs, more than ever, to be the subject of a multidisciplinary discussion within the Heart Team [60-64].

Futility of TAVI?

The identification of patients in whom a TAVI may be futile also remains an open question in 2018. Classically, futility is defined as a death or lack of functional improvement in the short term (6 to 12 months). The potential futility of TAVI is mainly evoked in patients with extreme fragility, chronic renal failure or chronic obstructive pulmonary disease (COPD). In a study based on more than 300 consecutive patients, about one-third of whom had COPD, was observed that a TAVI had proved futile in more than one third of the cases. This excess mortality was also observed in other studies. The full clinical control is expected to help Heart Team members distinguish patients for whom symptomatic benefit is expected from patients for whom the intervention will not improve quality of life or independence. Pragmatically however, Mok et al. propose performing a six-minute walk test and identify a distance of less than 170 meters as a good predictor of futility [65, 66].

Heart team

Finally, remember that according to the new recommendations, to define in a personalized way the most appropriate treatment for a patient, each case must be assessed in a multidisciplinary way by specialists, the Heart Team, already mentioned several times in this article. If the concept was developed long ago in the treatment of coronary heart disease, its use for the treatment of valvulopathies is recent and follows the advent of percutaneous therapeutic approaches. According to the European recommendations, this Heart Team brings together a panel of specialists, such as a cardiac surgeon, an interventional cardiologist, a non-invasive cardiologist, imaging specialist, a radiologist, a cardiac anesthesiologist and a geriatrician to be able to establish an individualized therapeutic project, taking into account all the dimensions (anatomical, functional and human) of the pathology of the patient with the ultimate goal of establishing if the patient is a candidate for a conventional surgical treatment or if his case is more a transcatheter or medical approach.

Conclusions

1. There is no doubt that TAVI is superior to medical treatment in inoperable patients.
2. In operable patients, TAVI is not inferior to surgery in high-risk patients and in intermediate-risk patients and even superior to surgery when a transfemoral route is feasible.
3. It is also important to consider comorbidities not taken into account by their risk scores and geriatric status in order to make the best decision for our patients.
4. The extension of indications to low-risk patients is already being evaluated ("PARTNER 3" and "CoreValve low risk" studies) and the results are expected in 2019-2020.
5. In parallel, it is important to collect as much information as possible about the durability of percutaneously implanted bioprostheses.

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Author's ORCID iD and academic degrees
Oleg Calenici – https://orcid.org/0000-0002-1600-9558, MD, PhD.

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Acupoint embedding therapy

[1,2]Olga Ignatov, [2]Oleg Pascal, [1]Viorel Nacu

[1]Laboratory of Tissue Engineering and Cell Cultures, [2]Department of Rehabilitation
Nicolae Testemitsanu State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova

Authors' ORCID iDs, academic degrees and contributions are available at the end of the article

*Corresponding author: olga.ignatov@usmf.md
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Abstract

Background: Peripheral nerve trauma remains a major cause of motor disability, at the same time functional restoration after treatment continues to show modest results. Acupoint embedding therapy is a type of acupuncture treatment in which different biodegradable materials are inserted into specific points for long-term stimulation. It has a good analgesic effect in chronic pain, and it is considered a cure for many diseases. Different biodegradable materials have been developed and widely used. Catgut has a good biodegradability and low price, but it could cause infections and having unstable chemical properties had been limited in clinical use. Such synthetic materials as polylactic acid and polyglycolic acid present low-cost, good biodegradability and biocompatibility compared with the catgut. However, their poor hydrophilicity and cell adhesion limited their therapeutic efficacy. The ideal embedding materials are required to be safe, non-toxic, biocompatible, and to have excellent swelling and biodegradation behaviors. Acupoint embedding therapy can be a promising treatment method of peripheral nerve disorders.
Conclusions: Acupoint embedding therapy is an invasive treatment which can prolong point stimulation, reduces the frequencies of pain and psychological fear of patients. It seems to be a promising method of neuropathy treatment. The properties of the filaments for acupoint embedding therapy can be improved by surface modification technologies.
Key words: acupuncture embedding therapy, neuropathy.

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Introduction

Acupuncture is generally held to have originated in China, being first mentioned in documents dating from a few hundred years leading up to the Common Era. Sharpened stones and bones that date from about 6000 BC have been interpreted as instruments for acupuncture treatment [1, 2].

The first medical description of acupuncture by a European physician was by Ten Rhijne, in about 1680, who worked for the East India Company and witnessed acupuncture practice in Japan [3, 4].

In 1960s, there appeared a treatment method by implanting absorbable materials (e.g. catgut) substituting for filiform needles into the acupoints, which realized long-time needle retaining and also avoided the danger of filiform needle retaining. This method was then termed acupoint thread-embedding therapy. During recent years, by constant improvement of embedding apparatus and materials, this method has gradually evolved to be micro-invasive thread-embedding therapy [5].

Traditional acupuncture. Acupuncture is a medical intervention in which fine needles are applied to specific parts of the body, called acupuncture points (or acupoints) and penetrated through the muscular or other subcutaneous layers. According to the theory of traditional Chinese medicine, acupuncture modulates the flow of Qi and blood through the meridians and restores the balance of the five organs to maintain homeostasis [6]. Each acupoint has its own specific therapeutic actions. They are presumed to be pathophysiologically associated with and possibly reflect the status of visceral organs and systemic conditions, and thus the stimulation of specific acupoints may evoke the responsiveness that controls the unbalanced internal milieu and improves body symptoms. Acupuncture stimulation is given right on the acupoint or a nearby affected area "*ashi point*" for the treatment of local symptoms, whereas distal acupuncture stimulation is applied to treat diseases in the internal organs and systemic abnormalities [7].

In manual acupuncture, an acupuncturist penetrates the skin with a metallic needle and manipulates it by rotating in one or both directions or lifting and thrusting [8]. Between 5 and 15 needles are used in a typical treatment, with the point combinations varying during a course of sessions [9]. It is known that during acupuncture practice, acupuncturists experience a special touch sensation perceived as heaviness, tenseness, or terseness, and patients perceive feelings of numbness, heaviness, soreness, and distention around the site of needle stimulation. These are called *deqi* sensations. Clinical data further indicate that patients frequently feel *deqi* sensations spreading to other parts of the body [10, 11].

In electric acupuncture, a small electric current is applied to pairs of acupuncture needles [8]. To maximize therapeutic effects, acupuncture is usually practiced first by applying

manual acupuncture to evoke *deqi* sensation and followed by electrical stimulation for 15–20 minutes [10]. Traditional acupuncture therapy has the advantages of safety, validity and non-toxic side effects, but with and unexpected short function time and frequent operation [12].

Among acupunctures, there mainly are filiform needles, electric needles, hydro-needles (small dose point injection), fire needles, warm needles, skin needles (including plum-blossom needles), ear needles, prick blood-letting therapy and many others [13].

According to the World Health Organization, acupuncture has been shown to be an effective alternative or complementary treatment to 28 diseases, symptoms, or conditions [14].

Peripheral neuropathy is broadly defined as damage of the peripheral nervous system caused by a primary lesion or dysfunction [15]. The recovery of peripheral nerve injury is a long and slow process. This may be because of the period of time needed for neural regeneration and functional reconstruction, but treatment methods may also contribute to the delay in recovery [16, 17]. Peripheral neuropathy, particularly when it involves the large nerve fibers, is ideally suited for a localized structural needling approach. Needles placed in close proximity to a nerve stimulate the nerve fibers directly, with an electric current or a physical stimulus from manual acupuncture [18].

He et al. note that nerve injuries affect the metabolic microenvironment. Citing an example, they note that sciatic nerve injuries reduce acetylcholinesterase activity in the lumbar spinal cord microenvironment. This causes neuronal cell death thereby impeding nerve repair. The researchers note that acupuncture counteracts this effect citing that it successfully increases "acetylcholinesterase expression in spinal cord tissue after peripheral nerve injury". As a result, this may be an important mechanism by which acupuncture promotes the healing of peripheral nerves [19].

Lu et al. revealed that acupuncture and electroacupuncture could accelerate the maturity of regenerated nerves with larger mean values of axon number, endoneurial area, blood vessel number, and blood vessel area as compared with the controls [20].

Acupuncture is an established adjuvant analgesic modality for the treatment of chronic pain, and it is considered a cure for many ailments and disorders [21]. It is thought to stimulate inhibitory nerve fibers for a short period, reducing transmission of pain signal to the brain [22]. Acupuncture treatment activates endogenous analgesic mechanisms [23], causing secretion of endorphin which is an endogenous opioid [24] and triggering release of adenosine [25], producing a rapidly effective analgesic action. Extensive research has shown that acupuncture analgesia may be initiated by stimulation of high-threshold, small-diameter nerves in the muscles [26].

Han et al. found that acupuncture can promote release of neurotransmitter such as 5- hydroxy tryptamine and in addition it generates neuropeptide through electrical stimulation of different frequencies that has significant effect to pain reduction [27]. Park et al. speculate that acupuncture stimulation may trigger the responsiveness of sensory receptors and generate neural activity in its own specific way, which may be encoded in the cerebral cortex and autonomic neuronal center and exert its effects on regulating inflammation. Future investigations to explore whether acupuncture-specific vagal activity exists and acts on cells in target organs will be of great importance to gain insights into the mechanistic basis of acupuncture [7].

Acupuncture may elicit vegetative reflexes, thereby changing the flow of blood and enhancing functional properties of connective tissue and organs [28]. Litscher et al. showed that acupuncture may increase blood flow in the limbs [29]. Increased blood flow to the vasa nervorum and dependant capillary beds supplying the neurons [30] may therefore contribute to the immediate effect of acupuncture [28]. Over some time, these may contribute to nerve repair with measurable improvement of axons or myelin sheaths after 10 treatments. Local and central effects on vascularization may thus represent combined causes for regeneration [31, 32]. Acupuncture could regulate multiple molecules and signaling pathways that lead to excitotoxicity, oxidative stress, inflammation, and neurons death and survival and also promote neurogenesis, angiogenesis, and neuroplasticity after ischemic damage [33].

Risk factors for complications of acupuncture:

– For pregnant women, avoid needling points on the abdomen and lumbar region, and certain points known to cause strong sensations;

– Hemophilia can affect clotting factors;

– Advanced liver disease could compromise production of clotting factors;

– Patients taking blood thinners could bleed for longer periods;

– Patients with HIV infection or immunocompromised patients are at increased risk of opportunistic infections;

– Patients with diabetes are subject to poor wound healing; neuropathy can reduce sensory ability, leaving them at increased risk of undetected infection;

– Patients who have had transplants often take immune suppressants that make them prone to infections;

– High-dose steroids suppress the immune system;

– Open wounds increase risk of infection;

– Hypoglycemic, nervous, or very fatigued patients might faint.

Potential adverse events associated with acupuncture are: fainting during treatment, nausea and vomiting, increased pain, diarrhea, local skin irritation, headaches, sweating, dizziness, aggravation of symptoms, needle breakage.

Rare complications include: pneumothorax, spinal cord injury, septicemia, punctured organs, convulsions, argyria [34].

The most adverse effects associated with acupuncture are minor and serious complications are rare. Patients should be forewarned of potential common, though minor, adverse effects. Needle penetration might cause pain and bleeding. Reports of fainting remind practitioners that patients might

be better off prone than sitting during treatments. Practitioners should keep some potentially fatal effects in mind when patients report shortness of breath, pleuritic chest pain, or fever after acupuncture treatment. All acupuncture practitioners should have proper training in techniques and safety [34].

Acupoint embedding therapy

In traditional Chinese medicine, catgut-embedding therapy has been used for the treatment of several diseases such as musculoskeletal pain, obesity, chronic urticaria, perimenopausal syndrome, depressive neurosis and others [35]. It is special type of acupuncture that inserts medical threads into skin, subcutaneous tissue or muscles at specific points [36]. Although acupoint catgut embedding therapy is considered an invasive treatment from a western medicine perspective, it has attracted considerable attention from clinicians in China due to its easy operation and durable stimulation, and has been widely used in recent years. Suture buried in acupoints can produce persistent stimulation on the basis of selecting sutures and acupoints according to deficiency and excess, which aims to regulate *yin* and *yang*, dredge the channels and collaterals, and reinforce the antipathogenic *Qi* and eliminate the pathogenic *Qi*. It has dual rapid and continuous action for chronic diseases. It is the combination of a variety of therapies (acupoint blocking, acupuncture, pricking, the after effect of tissue injury, needle retention). The intensity of stimulus would be changed with the time and the special needles and sutures can produce stronger efficacy than conventional acupuncture on controlling mind (*shen*) to facilitate *Qi* flow and dredging the meridian to regulate *Qi* and blood [37].

The effects of acupoint catgut embedding therapy in Western Medicine are similar to those of manual acupuncture. It provides both physical and chemical stimulation [38, 39].

The absorbable surgical thread, a foreign protein, can induce allergic reactions and the combined effects of proteolytic enzymes and macrophage action against the absorbable surgical thread may strengthen and extend the acupoint stimulation for 15–20 days as a consequence of the mild irritation in subcutaneous tissue, inducing a more persistent and potent physiological stimulation produced by the suture at the acupoints [39]. Acupoint embedding therapy improves body's nutrient metabolism and promotes blood circulation [37].

By using the specially made disposable embedding needle, the operation of micro-invasive thread embedding can be completed swiftly and conveniently in the way of injection. The embedding needle has a handle to hold and allows rapid inserting by only a hand. The mechanical spring ensures successful embedding of the thread. The marks on the needle body make it easy to control the inserting depth. The whole operation only takes 5-10 min, as only the thread not the needle needs to be retained. The stimulation produced by the retained thread can last for 1-2 weeks, during which the patients can move freely without any influence on their life. Therefore, this method can be applied in clinic safely for patients' convenience and less pain [40].

Patient should be informed about the local pain during operation and the postoperative reaction.

After the insertion, may occur such reactions as: a) the local skin will manifest redness, swelling, hotness and pain of aseptic reaction; b) hematoma may occur due to the needling injury and thread; c) general response with a fever of about 38^0C in 4-24hs, or even persistent high fever [37].

Thread-embedding acupuncture is a new subtype of acupuncture treatment developed from catgut-embedding therapy [35]. Different biodegradable materials have been developed and widely used. They are divided into natural and synthetic types according to material sources. Both of them have advantages and disadvantages [41]. For instance, natural embedding material is inexpensive and plentiful, but it also easily leads to an infection reaction due to poor biocompatibility. Hence, it has a higher applied risk due to the instable quality [42]. In contrast, synthetic embedding materials, such as polyglycolic acid [43] and polylactic acid [44], offer an excellent biodegradability and biocompatibility, and have a stable property through the whole implanting period. But their poor hydrophilicity and cell adhesion, inevitable large dimensions for producing a lasting effect largely affected their therapeutic efficacy in clinical application [45, 46].

There is a number of uncertainties, including the spinning speed, drawing temperature and stretching ratio, which affect compression behaviors in the preparation process of acupoint embedding monofilaments [47]. In addition, rigid acupoint embedding monofilaments will stimulate the subcutaneous tissue of the human body, resulting in a greater sense of pain for patients in clinical application. In contrast, soft acupoint embedding monofilaments may lead to difficulties in the embedding operation [48]. However, there is still a lack of relative study and a unified standard for a test method for monofilament compression behaviors [49].

The ideal embedding materials are required to provide properties such as safety, non-toxicity, good biocompatibility and absorbability [50, 51]. Antibacterial properties are essential in the prevention of infections [52, 53]. Also, embedding materials should provide enough mechanical properties to be implanted into the body and support peripheral nerve tissue [54]. Through good swelling behavior they are able to produce enough stimulus degree *in vivo*, yet retain a relatively small dimension *in vitro* and avoid the significant trauma [55]. Favorable hydrophilicity is also important for embedding material to adhere cells and work well in the human body [56].

Discussion

Catgut is a protein fiber of biological origin, that is derived from the small intestines of animals, mostly sheep or oxen [56]. Not many studies have investigated the mechanism of catgut implantation at acupoints. Li et al. explored the probable mechanism focusing on neurogenic inflammation [57]. Some studies have demonstrated that nerve stimulation could induce leukocyte activation and plasma

extravasation, which is termed neurogenic inflammation [58, 59]. The mechanisms involve the regulation of nerve conduction, signal pathways, hormone level, protein expression, oxidative stress level, and structure restoration. The treatment generally regulates some specific biochemical factors to influence particular signal pathways, eventually controlling the apoptosis and proliferation of cells and repairing the damaged structure [60]. The mechanism of acupoint catgut embedding regards catgut as a type of heterogeneous protein, after inserting it into acupoints, its process of softening, decomposition, liquidation and absorption can effectively promote and enhance the nutrition metabolism, stress ability of bodies, vascular permeability and blood circulation [61]. Areas of catgut suture degradation contain dense accumulation of macrophages, lymphocytes, and foreign body giant cells. After complete absorption, these are replaced by a dense mass of macrophages [62]. Most studies indicate that catgut sutures are completely absorbed between 35 and 60 days [63]. However, the safety of catgut embedding has not yet been established, since it can cause various immune reactions, including allergic reactions and a subcutaneous nodule [64].

Polydioxanone (PDO) has been used in acupoint embedding therapy devices in Korea. It is a synthetic monofilamentous polymer made of polyester or a polymer of polydioxanone. PDO retains 50% tensile strength after 4 weeks, takes 180 days to be absorbed, and also has low tissue reactivity [65]. In Korea, the embedding of PDO thread at certain acupoints is widely used in the clinical practice for the treatment of chronic musculoskeletal pain. Nevertheless, the current level of evidence supporting the efficacy of embedding with PDO thread for patients with chronic musculoskeletal pain is insufficient [66]. Only case studies on the application of PDO embedding therapy for the patients with chronic low back pain treatment [67], shoulder pain [68], and osteoarthritis of the knee [69] have been published. In addition, no randomized clinical trials using PDO thread embedded as a sham-controlled intervention have been reported [70].

Polyglycolic acid (PGA) has held great promise for various biomedical applications in human tissue due to its advantages of being easily available, low cost and having excellent biodegradability and biocompatibility [71]. PGA monofilaments prepared by melt-spinning technology for acupoint embedding therapy materials have exhibited sufficient supply, good formation capacity and versatility in terms of surface functionalization [72, 73]. The PGA monofilament is considered to be a highly promising biodegradable material for ideal acupoint embedding therapy material preparation [74, 75]. PGA is broken down by hydrolysis into its respective acids and alcohols. It tends to lose mechanical strength rapidly, over a period of 2–4 weeks after implantation [76]. Some shortcomings were discovered and they may increase the risk of PGA implantations, such as insufficient hydrophilicity and cell adhesion caused by the relatively smooth surfaces [45]. These features are believed to be the main contributors to the unfavorable characteristics [77].

Polylactic acid (PLA) is one of the highest consuming bioplastics in the world. It is an aliphatic polyester obtained from renewable sources such as corn sugar, starch, potato, and sugarcane. PLA has 37% crystallinity, elongation at break 30.7%, glass transition temperature of 53°C, and a melting temperature ranging between 170–180°C [78]. It is used in the biomedical area, and has also begun to be applied as an embedding material for curing osteoporosis [79] and pseudo-myopia [80] and other diseases. However, its long degradation time (about 2 years) and poor hydrophilicity have limited its further application in acupoint embedding therapy [81].

Polylactic glycolic acid (PLGA) is polymerized by 9 polyglycolic acids (PGA) and one polylactide acid (PLA). It is simple in composition without bioproteins, and is now widely used as embedding material for its proper rigidity. When implanted in the acupoint of human body, it can produce a long-time stimulation which is similar to that produced by a filiform needle [82]. Histologic examination showed that the PGLA sutures were absorbed within 90 days [83], which might be the underlying mechanism of persistent analgesic effects [84]. PGLA has good biocompatibility, and no adverse reactions have been reported. Hydrophilicity, cell adhesion and degradation properties can be improved by surface modification technologies [85].

Surface modification technologies

Currently, there are numerous surface modification technologies, such as dip-coating, ultrasound, chemical vapor deposition, ion beam injection and surface graft polymerization, that can be applied to biomedical materials [86, 87]. Wang et al. attempted to use ultrasound treatment to modify PGA and polylactic-glycolic acid (PLGA) fibers, and the results suggested that both PGA and PLGA fibers achieved better hydrophilicity and cytocompatibility, while the tensile strength of PLGA increased and that of PGA changed little [88]. Among a variety of surface modification technologies, cold plasma modification has been regarded as one of the simplest and highly effective methods to modify biomaterials. It can promote the formation of new oxidized functional groups by introducing potential activation sites on their surfaces, and modify the remaining original properties of materials to a great extent without damaging their outermost surfaces [89, 90]. Song et al. point out that the photo-degradation, thermal, and microbial biodegradable properties of the PLA films can be significantly improved by plasma modification [91].

It is reported that solution dip-coating is one of the most widely used processes in textile manufacturing and the simplest functionalization technique for material surfaces, and chitosan is a promising natural compound, which possesses a prospective future due to its advantages of non-toxicity, antibacterial properties, biocompatibility, biodegradation and swelling properties [92, 93]. Chitosan is a linear, high molecular weight heteropolysaccharide [94], consisting of N-acetyl- glucosamine and N-glucosamine units [95]. With its abundant reserve, chitosan is the second most important natural polymer globally (the first is cellulose) and has been

widely extracted from marine arthropods (prawns, crabs, shellfish, etc.) [96]. Hence, numerous chitosan coating applications on tissue engineering areas have been reported. Dubnika et al. used chitosan as an antibacterial agent to functionalize scaffolds and achieved a significant effect [97]. Niekraszewicz et al. used chitosan to modify polypropylene (PP) mesh and achieved good results in terms of mechanical and chemical properties; the biological purity was also improved [98]. Umair and colleagues reported that the PGA suture using N-halamine-based chitosan agents, its antibacterial efficacy was enhanced and could kill both *E. coli* and *S. aureus* bacteria within 15 min of contact time [99]. However, there is still a lack of detailed research about chitosan coating methods of monofilaments and existing studies prefer to target the biocompatibility, proliferation capacity, etc. Meanwhile, there are few reports on the self-swelling behaviors of embedding materials improved by chitosan coating [100].

Conclusions

1. Acupoint embedding therapy is an invasive treatment which can prolong point stimulation, reduces the frequencies of pain and psychological fear of patients and visits to the doctors.

2. The acupoint embedding therapy seems to be a promising method of neuropathy treatment.

3. The ideal embedding materials are required to provide properties such as safety, non-toxicity, good biocompatibility, excellent swelling and biodegradation behaviors.

4. The properties of the filaments for accupoint embedding therapy can be improved by surface modification technologies.

5. It will be great if the embedded material has also an antibacterial or active factor releasing effect which can be achieved by dip coating with chitosan or other substances.

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Authors' ORCID iDs and academic degrees
Olga Ignatov – https://orcid.org/0000-0003-2911-3239, MD
Oleg Pascal – https://orcid.org/0000-0002-4870-2293, MD, PhD.
Viorel Nacu – https://orcid.org/0000-0003-2274-9912, MD, PhD.

Authors' contributions
OI designed the trial, wrote the fist draft of manuscript. OP interpreted the data. VN revised the manuscript critically. All the authors revised and approved the final version of the manuscript.

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General provisions on medication errors committed by pharmacists

Nicoleta Cheptanari-Birta

Vasile Procopisin Department of Social Pharmacy, *Nicolae Testemitsanu* State University of Medicine and Pharmacy Chisinau, the Republic of Moldova

Author's ORCID iD, academic degree and contribution are available at the end of the article

Corresponding author: nicoleta.cheptanari@usmf.md
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Abstract

Background: Healthcare professionals are not fully aware of the harm caused by medication errors in terms of patient discomfort and economic burden. The problem being presented, we propose the analysis of the specificity of the pharmaceutical activity and the research of the errors committed by the pharmacists for the elaboration of the recommendations for their prevention.
Material and methods: This research is a meta-analysis in which it quantitatively combined the results from previous studies to obtain a summary value. The research was carried out in 4 stages: identification, selection, extraction and data analysis.
Results: Studying the specialized literature we identified great gaps in the knowledge of the actual data about the frequency of medication errors and their classification. Quality research is needed to determine the effectiveness of the following interventions: implementation of efficient and qualitative tools that will contribute to reducing medication errors; educational interventions regarding risk factor management; developing an anonymous system for reporting medication errors; review of the schedule, workload and conditions under which pharmacists work.
Conclusions: Medication errors require clear and unambiguous definitions where patients, doctors, manufacturers, and regulators will understand each other. The pharmacists' ethics manifests itself in honest acknowledgment of their mistakes, because in this profession, as in no other profession, the slightest inaccuracy will lead to serious consequences and can be fatal for the patient.
Key words: medication errors, pharmacists, risk factors, classification.

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Introduction

Health is one of the main indicators of life quality and the essential factor of sustainable development of society. As in the case of other countries, the health care system in Moldova must respond to new needs, appeared from demographic and socio-cultural changes, globalization process and rapid progress of medical technologies.

Pharmacists are the most often visited and accessible members of the health care team, being visited by sick and healthy people. For example, in the countries of the European Union, the 107.000 pharmacists serve 343.3 million of the population. Of this number about 17 million visit the pharmacy daily [1].

The pharmacist undertakes his/her own responsibility for each prescription, which he/she must check. He is the professional of health making up the last link in the way of the drug, guiding the patient against its misuse. The guarantee of quality and safety brought by the pharmacist is a true foundation that he consents the preparation and releasating of the drug [2].

Purpose. The problem being presented, is proposed to be analyzed to highlight the specifics of the pharmaceutical activity and research the errors committed by pharmacists to develop recommendations for their prevention.

Material and methods

This research is a meta-analysis in which the results from previous studies have been combined to obtain a summary value. The research was divided into 4 stages: identification, selection, extraction and analysis of data.

At the first stage, were identified all the articles corresponding to the subject under investigation. After collecting the articles followed the selection of true information from them and the extraction of relevant data. Finally, all the data were analyzed and conclusions were formulated.

Results and discussion

Studying the literature we have identified large gaps in the knowledge of current data about the frequency of medication errors and their classification. Quality researches are needed to determine the effectiveness of the following interventions, which would help to prevent medication errors:

- Computerized systems through which the doctor can complete and transmit the pharmacy recipes online and who can also check according to drug allergy and drug interactions, and, as well and as quickly inform about warnings regarding the correlation of prescriptions with the patient's condition. This system will make much easier the pharmacist's work, and due to

its speed, overloads of the pharmacist will be avoided;
- Educational interventions according to the management of factors which contribute to the occurrence of medication errors;
- Developing an anonymous system for reporting medication errors;
- Improving the level of professional knowledge of pharmacists;
- Double check by pharmacists of prescription drugs before release;
- The involvement of pharmacists in reporting the factors that cause them to make errors;
- The review of the program, the volume of work and the conditions under which pharmacists work.

The activity of the pharmacist in the open-circuit pharmacy is that of counseling the patient in mild conditions that do not require consultation with the doctor and guidance to the doctor where the gravity of the situation imposes advice on how to administer the medication recommended by the doctor [3], warning of potential adverse reactions and side effects (for example: gastric discomfort / gastrointestinal ulcer in the case of NSAID, intolerance to certain substances, possible teratogenic effects when administering certain drugs to pregnant women, especially in the first trimester of pregnancy), information on combinations of medicines or foods that may decrease the effectiveness of medication (for example: concomitant administration of oral contraceptives with tetracyclines, vitamin C, combination of tetracyclines with dairy foodstuffs or salts of Ca2+, Al3+, Fe2+, Bi3+, Mg2+ – oral antacids), warning on some associations contraindicated or those done with precautions (for example: combination of NSAID at patients in treatment with oral anticoagulants, or at the patients with asthma with/without allergic component, association of beta-blockers (non-selective propranolol) at patients with hypoglycaemic therapy or selective at persons in treatment with anti-asthmatic or antidepressant medicinal products (for antidepressants, only for lipophilic beta blockers), the usage of caffeine-containing drinks or OTC medicines in gout patients) [4].

The pharmacist who works in an open-circuit pharmacy can play an important role in education of the population both in terms of OTC medication, as well as in the treatment recommended by the doctor, showing the professionalism and promptness in providing the information as clearly as possible, in a language accessible to the patient [5]. His role is not only to release the drug, but also to ensure that the information he transmits with its release has been fully understood, that the treatment scheme will be respected and as in the case of undesirable or serious manifestations the patient will contact the doctor or pharmacist as soon as possible [6].

All these and many other activities are part of the specifics of pharmaceutical activity that is always at risk. At first, the main risk to which pharmacists were exposed was related to the usual threats lurking in a business (thefts, fires, etc.), and also to negligence regarding errors in the release of the recipes. Now, modern pharmaceutical practice must take into account new risks, related to the usage of technologies and electronic data transmission, patient counseling and requirements for evaluating the administration of medicines as well as confidential health information. Pharmaceutical practice evolves, and with it increases the risks related to the changing environment and objectives. Pharmacists should be conscious of the inherent risks of the provision of medical products and services and develop risk management strategies to counteract them [7]. All these risks have negative consequences of medication errors that can have a negative impact on patients' lives.

The problem of medical and pharmaceutical errors has been and remains one of the most important in the field of healthcare in many countries of the world. One of these problems was the terrible "tragedy of thalidomide" (from 1956 to 1962), when after thalidomide prescribing at pregnant women were born up to 12 thousand children with congenital malformations [8] and it was after this time that special pharmacovigilance services were created for the first time in several countries of the world to identify and prevent complications of drug therapy.

In the Republic of Moldova studies have not been conducted that reflect the statistics of medication errors, but the analysis of similar data from other countries shows that:
- in the United States of America, the pharmacists make 4-12 mistakes out of 100 cases of drugs delivery, about 87% of errors are caused by incorrect reading of a recipe, the confusion on behalf of medicinal products and packaging design [9];
- in community pharmacies in the United Kingdom, the errors of drugs releasing constituted 0.01-3.32% [10];
- in Denmark were identified up to 0.6% of prescriptions containing medication errors, but 8.7% of them can lead to deaths [11].

According to Professor N. Schaad of the University of Medicine in Geneva, the medication errors represent any mistake in prescribing, releasing or administration of a medicinal product, whether these errors lead to negative consequences or not [12]. Practically it is an action made incorrectly or due to ignorance, caused by a miscalculation, writing, speech, law or failure to perform an action that was planned, as well as using a wrong action plan to achieve a goal.

Cognitive psychologists believe that errors, lapses are the price we pay for superior brain function and those errors are inevitable. Ernest Mach (1838 - 1916) was saying "knowledge and error flow from the same mental source, only success can differentiate them"[13].

In 2013, Ivan Anosov, an employee of the Department of pharmaceutical Management and Economics of the Friendship University of Russia [14], made a research for identifying the typical mistakes that pharmacists commit in their practice. This study classified the errors as follows:
- the error associated with the name of the drug – 34.4%;

– releasing of a wrong medicinal product – 22.1%;
– releasing children's medicines to an adult patient and vice versa – 15.3%;
– the error in calculating the dose, the concentration of the drug – 10.4%;
– the error in replacing the drug with an analogue – 9.8%;
– the recommendation and drug releasing which are not in accordance with the indications – 8.0%.

Regarding the last two types of errors mentioned above, was made a study in the Republic of Moldova in 2015 on *the Ethical promotion of medicines:* current approaches and regulations [15], in which 1000 people were questioned. At the question "*Did the pharmacist suggest you should buy a drug other than the one recommended/prescribed by the doctor?"* 22% of respondents answered: "Yes, very often"; 54.4 % of respondents answered: "Yes, sometimes"; 22.4 % of respondents answered: "No".

Based on the survey we attest the existence of the situations when pharmacists suggest buying other medicine than the one recommended/prescribed by the doctor. The changing of the doctor's option by the pharmacist can be based on various reasons: the lack of the product in the pharmacy, the encouragement of sales of a particular drug of a particular pharmaceutical company, etc.

At the question "Did t*he pharmacist insist to buy in addition to the medicine/s requested some other medicine/s?" 15*.4 % of respondents answered: "Yes, very often"; 28.2 % of respondents answered: "Yes, sometimes"; 56.2 % of respondents answered: "No". The majority of respondents said that the pharmacist did not insist on other drugs besides those requested. However, there are also respondents who have dealt with proposals from the pharmacist on the purchase of an add-on drug.

There is a difference between the attitude of a person who is not in the pharmaceutical field and the attitude of a pharmacist to his professional mistakes [16], and that is:

1. The pharmacist has a tendency to objectify the source of errors. His own thinking way, like that of a doctor, is dialectical and dynamic.

2. At patients, is often observed an opposite tendency – to see the omnipotent pharmaceutical science, but the source of errors is only in the pharmacist incompetence or in the unwillingness to help the patient. The thinking way of healthy and sick patients is logical-mechanical and statistical.

In order to understand how medication errors are committed and to develop methods of prevention their classification will be taken into account, what can be contextual, according to the mechanism of production and psychological. Contextual classification includes specific time, place, drugs and involved people. The classification according to the production mechanism examines how errors occur (e. g. by omission, repetition or replacement). However, is preferable the classification based on psychological theory because it explains the events and their description. According to this theory, medication errors can be classified as follows:

Category A: there are circumstances and events that are capable of causing errors;

Category B: an error has occurred, but the error does not affect the patient;

Category C: an error which affects the patient has occurred, but the error does not cause a harmful response to the patient;

Category D: an error affecting the patient has occurred and monitoring is required to confirm that the error does not cause a harmful response to the patient and / or the required intervention is required to exclude harmfulness;

Category E: an error affecting the patient occurred and may have contributed to or resulted in a temporary harmful response of the patient and no intervention was required;

Category F: an error that has occurred may have contributed or resulted in a temporary harmful response of the patient and was imposed an initial hospitalization or prolongation of hospitalization;

Category G: an error that has occurred may have contributed or resulted in a permanent harmful response of the patient;

Category H: an error that occurred may have required an intervention to support the patient's life;

Category I: an error that occurred may have contributed to or resulted in the patient's death [17, 18].

According to the mechanism of production, pharmaceutical errors are divided into (fig. 1):

➢ Errors produced by the pharmacist;
➢ Errors in which the pharmacist has not a fault.

All these errors can be committed by a huge range of drugs (20 thousand products), at the same time, too many medicines have similar orthographic names: tamiflu-theraflu, linex-linkas, somnil-sonmil, prostamol-paracetamol, ranitidine-remantadine, etc. As well as the similarities between the packaging of medicines (e.g. Cyston and Liv-52) or the same medicine, but in different doses. Pharmacists can also be influenced by tiredness, haste, overwork, illness, household problems, insufficient sleep or by conflicting patients. All these reduce the attention and can have negative effects on the patient's health.

Another cause of errors is the lack of qualified personnel. By the beginning of this year, there were about 1400 pharmacies in the Republic of Moldova, and their number is constantly increasing. About 80-90 specialists graduate from *Nicolae Testemitsanu* State University of Medicine and Pharmacy annually and only four out of ten of them work in pharmacies. According to the data, the number of non-professional employees in pharmaceutical organizations reaches 40% [20, 21]. On account of the acute deficiency of specialists, employers had to lower the requirements to the professional level of employees.

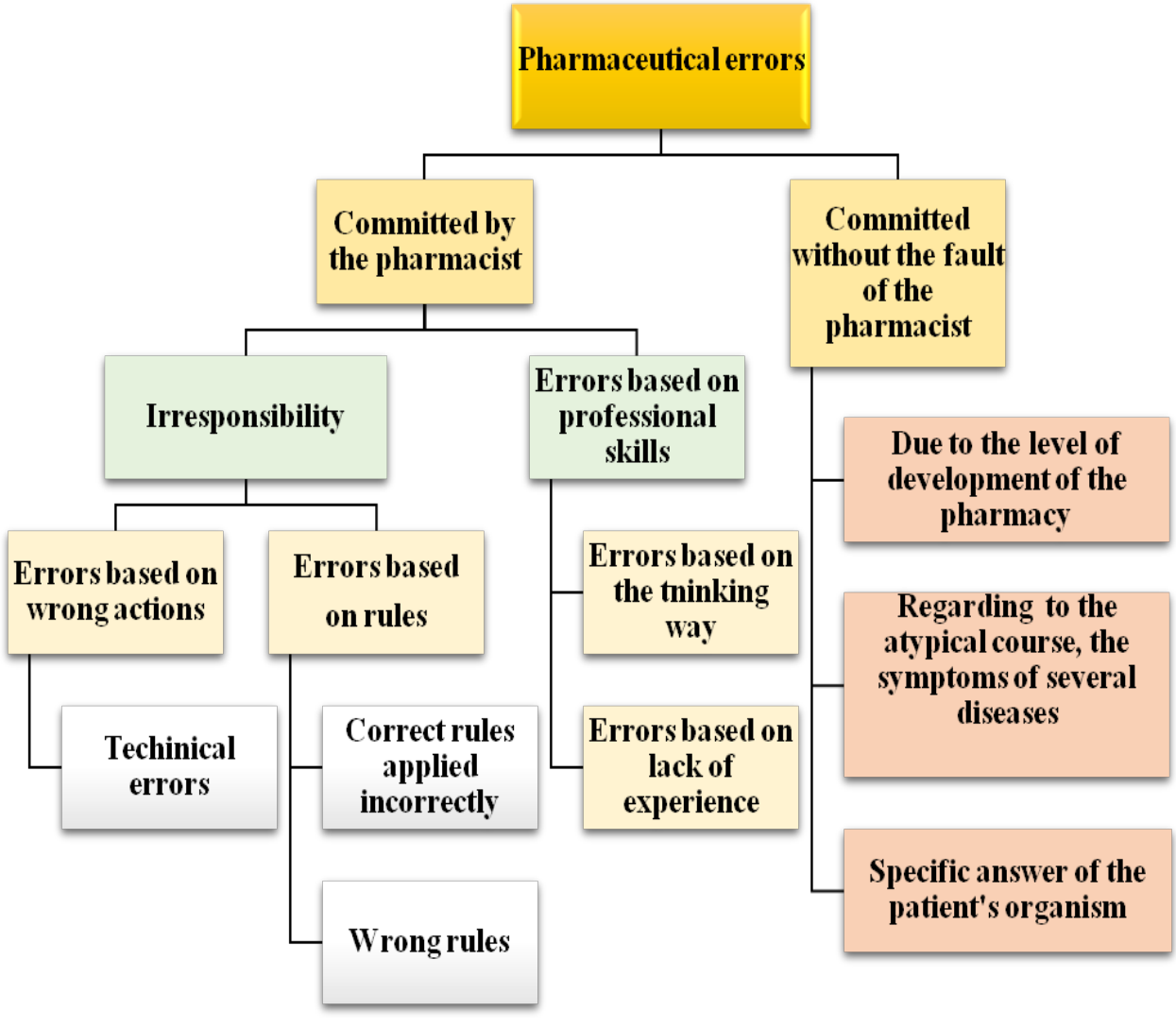

Fig. 1. The classification of medication errors according to the mechanism of production [19].

Conclusions

The medication errors require clear and unambiguous definitions, so that patients, doctors, manufacturers and regulatory authorities can understand each other.

The classification of medication errors based on how they occur, may suggest strategies that will help reduce their occurrence.

The ethics of the pharmacists is manifested in the honest recognition of their mistakes, because in this profession, as in no other profession, the slightest inaccuracy results in serious consequences and can be fatal for the patient.

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Author's ORCID iD and academic degrees
Nicoleta Cheptanari-Birta – https://orcid.org/0000-0002-1331-1161, PharmD.

Author's contribution
NCB conceptualized the idea, conducted literature review, wrote the manuscript, revised and approved the final text.

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Trauma scoring systems

*[1,2]Oleg Arnaut, [1]Dan Croitoru, [1]Ion Grabovschi, [2]Serghei Sandru

[1]Departmanet of Human Physiology and Biophysics, [2]*Valeriu Ghereg* Department of Anesthesiology and Intensive Care *Nicolae Testemițanu* State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova

Authors' ORCID iDs, academic degrees and contributions are available at the end of the article

*Corresponding author: oleg.arnaut@usmf.md

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Abstract

Background: Worldwide, traumas represent an actual theme of discussion. The recognition and interpretation of severe traumas are essential for choosing the right treatment strategy. There are two approaches to mark the patients with a high risk of unfavorable evolution and death. First, to use the terms as "major trauma", "severe trauma" and "polytrauma", without ability to stratify the patients according the severity of lesions inside categories mentioned above. Second, to implement the trauma scoring systems (anatomical, physiological or mixed), when a doctor uses a mathematical algorithm/model to calculate the risks for each trauma patient. At the same time, according to the articles found on PubMed/Medline, Web of Science, and EBSCO databases, there is no international consensus concerning the most accurate traumatic score. This article's goal was to revise the existing trauma scoring systems to highlight the potential scoring systems that in perspective can be validated in the Moldovan medical system.

Conclusions: Different traumatic scores are used worldwide (different continents, countries or regions) to estimate the severity of trauma patients in relation to the anatomical, physiological or combined criteria. All of them could be potentially validated for the Moldovan medical system. A part of these scores could be validated and compared to identify those ones that have the best determination, calibration and discrimination abilities to predict the outcomes for the local medical system. As a result, the coefficients from the mathematical equations belonging to the scores could be adjusted to the conditions of the national medical system of the Republic of Moldova.

Key words: trauma scoring systems, severe trauma.

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Introduction

Actually, traumas represent an actual subject at international scale, being the main cause of death in the world for the patients in the age category of 1-40 years [1, 2]. In the Republic of Moldova, according to the National Center for Management of the National Agency of Public Health, in the period of 2008-2017, traumas are on the 4th place in the list of causes of lethal outcome, constituting 8.1% (36889 cases) of all registered cases, being placed after the cardiovascular diseases (61%, 226195 cases), tumors (15.8%, 58518 cases) and digestive system diseases (10%, 36889 cases). The analysis of lethality structures according to the age showed that in the first year of life, traumas are placed on the 2nd place (30.3%) after the respiratory system diseases (57.9%). The lethality rate related to traumas is progressing along with the age and has its maximum incidence at the age of 18 years (81.3%), after that, it is decreasing, the lethality rate of traumas being 24.1%, and loses it predominance in the age category of 44 years and further, when the cardiovascular diseases are dominant (26.3%), being in decrease until 0% at the senile age category [3]. The recognition and interpretation of severe traumas is essential for choosing the right treatment strategy.

To describe the patients with a high risk of unfavorable evolution and also of death, there exists a series of terms like "severe trauma", "major trauma" and "polytrauma". The analysis of entries/documents in Web of Science database shows 24441, 19471 and 2813 entries for these notions, respectively. The terms "severe trauma" and "major trauma" are very similar, synonymic, but the criteria are not precise and fixed, the critical value of ISS (Injury Severity Score) or NISS (New Injury Severity Score) varies in different studies at the threshold of 16-17 points [4, 5 ,6]. The polytraumas represent the most unexplored and unresearched part of traumas, being a narrow notion compared to severe trauma and major trauma. There are a lot of definitions for polytrauma. In most of the sources, the criteria for polytrauma represents the anatomical scale ISS, the value of more than 15 being the threshold. At the same time, according to other authors, this value varies from 15 up to 26 and more [7, 8, 9]. In a study made in 1996, it was proven that the medical personnel's incompetence represents one of the causes of the errors in the usage of ISS for polytrauma diagnosis [10]. Another criteria used for polytrauma definition are at least two lesions in any topographical region and at least one of them is a threat for the patient's life [9]. According to the New Berlin Definition, proposed and validated in studies

with high evidence, the polytrauma is defined as severe lesions for at least 2 body regions, appreciated by AIS (Abbreviated Injury Scale) with a score of ≥ 3 being present at least one of the 5 physiological parameters (systolic blood pressure ≤ 90 mmHg, GCS ≤ 8, acidosis, coagulopathy and age ≥ 70 years) [11]. At the same time a series of scores and algorithms are created to assess the severity of traumas, but at the moment, as a study has shown, there is no international consensus in the articles found on PubMed/Medline, Web of Science, and EBSCO databases according the most efficient scale, many of them claiming different things [9, 11, 12], this situation being related to geographical factors and differences in the medical systems, particularities of demographic structure [10].

On the other hand, the Moldovan medical system doesn't use any trauma scoring system that was validated in order to evaluate the patient's risk of death and complications in case of trauma. Because of that, at the patient's evaluation there are disagreements on the prognostic, different scores often estimating the outcomes completely different. The solution for this problem includes a few stages as follows. First of all, we need to revise the existing trauma scoring systems that can be used in the Moldovan medical system. Secondly, to validate these scores for the Moldovan medical system and to elaborate the new trauma scoring systems. Lastly, the comparative evaluation of the trauma scoring systems is necessary in order to identify the ones that have the optimal ability (determination, calibration and discrimination) to predict the outcomes for the medical system of Moldova.

This article's goal is to accomplish the first task listed above, especially to revise the existing trauma scoring systems to highlight the potential scoring systems that in perspective can be validated in the Moldovan medical system.

Material and methods

We revised the articles in the PubMed archive using the HINARY system, overall 77 sources. For each score, we have mentioned their mathematic models and for some of them a calculation example. We did not mention the coefficients for equations because they are available in the cited sources. The information was classified in correlation with the parameters included in presented models (anatomical, physiological and mixed scales) and also with geographical distribution (different continents, different countries or regions).

Results

The scoring systems used to evaluate the severity of traumas can be classified into 3 categories: (I) the anatomical scores that take into consideration the anatomical injuries as a result of the traumatic event, (II) the physiological scores that are based on the clinical signs/measurements, (III) mixed, that enrolled both anatomical and physiological parameters.

Anatomical scores

All the scores from this category are derived from the AIS (Abbreviated Injury Scale) or ICD (International Classification of Diseases and Related Health Problems, https://icd.codes/icd10cm). Each lesion has its own certain score, attributed using the AIS or ICD dictionaries. Medical staff can apply the scores using the existing algorithms. For example, according to last edition of Abbreviated Injury Scale (AIS dictionary 2015), the comminuted tibial fracture is estimated by 3 points and according to ISS9.

Abbreviated Injury Scale (AIS) Derived Scores

AIS represents an anatomical score that appreciates by a scale that varies from 1 to 6 the severity of a trauma in a topographical region of the body by the following model: 1 – Minor, 2 – Moderate, 3 – Serious, 4 – Severe, 5 – Critical and 6 – Fatal [13]. The topographical regions considered in this score are: Head and neck, Face, Thorax, Abdomen, Limbs (also includes the pelvis), Exterior (burns, skin lesions etc.). More recently a term called MAIS (Maximum AIS) was introduced. It represents the highest AIS value for any body region [14]. For example, in case of traumas combination $AIS_{thorax} = 3$ and $AIS_{abdomen} = 4$, the MAIS value is 4.

Injury Severity Score (ISS) and New Injury Severity Score (NISS)

In the past decades, ISS and NISS were used widely for the evaluation of the severity of trauma. To estimate ISS, we have to use the following formula: $ISS = A^2 + B^2 + C^2$, where A, B, C are the highest AIS values present in each topographic region. It can vary from 0 up to 75. In condition if there is a topographical region with AIS = 6, ISS is automatically equal to 75 [15]. NISS in comparison with ISS, estimates trauma severity taking into account three maximal values of AIS, indifferent of the lesions localization [14]. For example, in case of trauma in 4 topographical regions $AIS_{abdomen} = 2$, $AIS_{head\ and\ neck} = 3$, $AIS_{head\ and\ neck} = 3$ and $AIS_{Thorax} = 5$, the NISS value will be higher ($NISS=5^2 + 3^2 + 3^2 = 43$) versus ISS ($ISS=5^2 + 3^2 + 2^2 = 38$). At the same time, according to the results obtained by clinicians from China, NISS is similar to ISS in predicting the outcome of the traumatic patients [2]. We suppose that such result can be explained by insufficient determination coefficient (40%-60%) in equations that use NISS or ISS [16, 17].

Logarithm Injury Severity Score (LISS) and Exponential Injury Severity Score (EISS)

LISS uses the natural logarithm of AIS as follows: $LISS = \ln(A_1)^{5.53} \times 1.7987 + \ln(A_2)^{5.53} \times 1.7987 + \ln(A_3)^{5.53} \times 1.7987$, where A_1-A_3 are the AIS values for the three most severe traumas. For example, a patient with $AIS_{abdomen} = 3$, $AIS_{thorax} = 2$, $AIS_{head\ and\ neck} = 4$, $AIS_{limbs} = 5$, will have $LISS = \ln(3)^{5.53} \times 1.7987 + \ln(4)^{5.53} \times 1.7987 + \ln(5)^{5.53} \times 1.7987 = 38.9716620395$. According to the results obtained by certain researches it has tendency to have better calibration and discrimination characteristics than NISS [18].

EISS is based as LISS on the most severe AIS scores that are used in the following formula: $EISS = 3^{A-2} + 3^{B-2} + 3^{C-2}$, where A, B and C are the highest values of AIS [19]. For example, a patient has $AIS_{head\ and\ neck} = 3$, $AIS_{thorax} = 4$, $AIS_{abdomen} = 2$ and $AIS_{limbs} = 5$, in this case $EISS = 3^{5-2} + 3^{4-2} + 3^{3-2} = 27+9+3=39$.

Anatomic Profile Characterization (APC)

APC is a scale that was proposed by Copes et al. According to APC algorithm a doctor has to take into consideration only the 3 most severe lesions according to AIS. The AIS scores are grouped in relation to region – A (AIS = 3-5 head, neck, brain and the spinal cord), B (AIS = 3-5 thorax), C (the anterior region of the neck with AIS = 3-5, the abdomen and pelvis with AIS = 3-5, the spine with or without the spinal column with AIS = 3, pelvic fractures with AIS = 4-5), D (the femoral artery with AIS = 4-5, collapse above the knee with AIS = 4-5, amputation above the knee with AIS = 4-5, the popliteal artery with AIS = 4, the face with AIS = 1-4, other traumas with AIS = 1-2). All of the conditions described above being classified based on ICD-9-CM, APC will be further on calculated using the following formula: $APC = M_0 + M_1 \times A + M_2 \times B + M_3 \times B^2 + M_4 \times C^2$, the used coefficients are: $M_0 = 4.0801$; $M_1 = -0.4914$; $M_2 = -0.2066$; $M_3 = 0.0161$; $M_4 = -0.0351$. D was excluded because in this case it wasn't influencing the survival predictability, but in some geographical regions it may be useful. The obtained value (APC) is considered in logistic regression formula as b and $P(survival) = e^b/(1+e^b)$ [20]. For example, we have a patient with $AIS_{abdomen} = 2$, $AIS_{Head\ and\ neck} = 3$, $AIS_{Upper\ limb} = 4$ and $AIS_{Thorax} = 5$, in this case $APC = 4.0801 - 0.4914 \times 3 - 0.2066 \times 5 + 0.0161 \times 5^2 - 0.0351 \times 0 = 1.9754$, further on, $P(survival) = e^{1.9754}/(1+e^{1.9754}) = 0.8781$, respectively, the chance for survival in this case is equal to approximately 87.81%.

International Classification of Diseases (ICD) Derived Scales.

Trauma Mortality Prediction Model (TMPM)

TMPM is an algorithm that takes into consideration the 5 most severe traumas ordered from the least severe to the most severe and also includes a binary variable that reflects the presence of the 2 most severe traumas in the same body region [46]. The survival probability is calculated using the following formula: $TMPM = C_0 + \sum^5_{i=1} (C_iI_i) + \eta S + \sigma I_1I_2$ where I_1-I_5 are the MARC values (Model-Averaged Regression Coefficient) for the lesions described in ICD-10-CM, written from the least severe to the most severe (I_1 being the most severe), the MARC values must be calculated *de novo* or extracted from a database, they are divided depending on gender, age and trauma mechanism, by the way, these 3 criteria determine the necessity to create 3 x 3 = 9 groups of MARC values (for example, MARC values senile patients of male gender with penetrant traumas will differ from the MARC values attributed to the pediatric patients of female gender with blunt traumas) , the method used to calculate MARC values is exposed in the original article that belongs to Glance L. et al., S is the binary value, equal with 1 if the 2 most severe traumas are present in the same body region, $C_0 – C_5$ are coefficients that have the following values: $C_1 = 1.4298$, $C_2 = 1.3942$, $C_3 = 0.5190$, $C_4 = 0.3981$, $C_5 = 0.8278$, $C_0 = -2.2104$, $\eta = -0.1059$, $\sigma = -0.7835$, $P(survival) = 1/\sqrt{(2\pi)} \int^x_{-\infty} (e^{(-t\wedge 2/2)})dt$, where t = TEMPT [21].

Injury mortality prediction (IMP)

The IMP derives from ICD-9-CM and is used to predict the probability of survival for a traumatic patient. The score considers the 5 most severe traumas and is calculated using the following algorithm:

$IMP = C_0 + \sum^5_{i=1} (C_iI_i) + C_6S + C_7I_1I_2 + C_8 \ln(NBR) + C_9 NBR^{0.382}$, where I_1-I_5 are the WADP values (Weighted Average Death Probability) for the 5 most severe traumas, the WADP values pot can be derived using a database or can be extracted from an existing one, S is a binary variable that is equal to 1 if 2 of the most severe traumas are located in the same topographical region, NBR is the number of topographical regions with traumas in a patient, C_0 - C_9 are the coefficients that have the following – $C_1 = 2.6352$, $C_2 = 2.3540$, $C_3 = 0.3164$, $C_4 = 0.2047$, $C_5 = 0.3681$, $C_6 = -0.3080$, $C_7 = -0.6582$, $C_8 = -1.7419$, $C_9 = 1.6154$, $C_0 = 9.0177$, $P(survival) = 1/\sqrt{(2\pi)} \int^x_{-\infty} (e^{(-t\wedge 2/2)})dt$, where t = IMP [22].

ICD Derived Injury Severity Score (ICISS)

ICISS is an ISS derived score. It was formulated based on ICD-9 [23]. It can be calculated using the following formula: $ICISS = SRR_1 \times SRR_2 \times \ldots \times SRR_n$ where SRR is Survival Rate Ratio, n – the number of lesions. Every lesion has a specific SRR value that varies from 0 to 1. Also, it varies depending on age groups, gender and trauma mechanism. Accuracy of this prediction model is based on the number of patients that were used to derive the SRR values [24]. As an example, we will show an ICISS calculated using SRR values designated for ICD-9 derived from the Florida AHCA database for the 1991-2009 period (http://personal.health.usf.edu/epracht/ICISS/) for a senile patient with closed clavicle fracture (code 810.00, SRR = 0.9075), open skull base fracture with laceration and contusion (code 801.6, SRR = 0.7000), closed mandible fracture (code 802.2, SRR = 0.8713). The ICISS (chances for survival) in this case = 0.9075 x 0.7000 x 0.8713 = 0.5534 (55.34%).

Physiological Scores

There are used different algorithms for functional reserves estimation that can serve as scores in case of severe trauma: GCS, MODS, RTS, SOFA, SAPS II, APACHE II, MPM II [25].

Trauma Early Mortality Prediction Tool (TEMPT)

This is a scale used to predict the survival chances using the following variables: Age (≥ 59.5 years), Systolic blood pressure (≥ 163.5 mm Hg), Creatinine (≥ 1.35 mg/dl), International Normalized Ratio (≥ 1.25), Partial thromboplastin time (≥ 31.40 seconds), Hemoglobin (≤ 12.75 g/dl), Platelets (≤ 224.5 103/μL), Base excess (≤ −4.35 mmol/l), Temperature (≤ 36.25 °C). Each of these criteria has a coefficient that is included in the formula: TEMPT = ∑(Variables x Coefficients). If b = TEMPT, then $P(survival) = e^b/(1+e^b)$ [26].

MGAP and GAP scores

MGAP estimates the chances for survival considering the mechanism of trauma (blunt/penetrating), GCS (Glasgow Coma Scale), age, systolic blood pressure. It is calculated using the following model: GCS (the value of GCS), systolic blood pressure (>120 mm Hg – 5 points, 60-120 mm Hg – 3 points, <60 mm Hg – 0 points), the mechanism of trauma (blunt – 0 points, penetrant – 4 points), age

(when <60 years – 5 points). MGAP = ∑(Variables). As a result, the patients are divided in three groups. High chances of survival (MGAP = 23-29), medium chances of survival (MGAP = 18-22) and small chances of survival (MGAP = 3-17) [27]. For example, a patient with penetrant trauma (4 pts), age of 49 years (5 pts), systolic blood pressure of 87 mm Hg (3 pts) and GCS = 13 has MGAP = 4+5+3+13=25 a respectively high chances for survival.

GAP derives from MGAP and is calculated similarly, but the mechanism of trauma is ignored. GAP = ∑(Variables) we define here: high probability of death group (GAP = 3-10 points), moderate probability of death group (GAP = 11-18 points), low probability of death group (GAP = 19-24 points) [28].

Kampala Trauma Score (KTS)

It is a score that was developed in Uganda to appreciate the severity of traumas, the main components are – age, systolic blood pressure, respiration rate, neurologic status (based on the AVPU scale), presence or absence of severe lesions. KTS can vary limits from 5 up to 16, to calculate the survival probability, it is necessary to create a local database based on the following model: P(survival) = (Number of deaths with the following score)/ (Total number of deaths), that must be made for each score individually, it will appreciate the survival probability percentage, the conclusion being made based on the previous cases [29]. An example for the KTS calculation – a patient with SBP of 107 mm Hg, respiration rate – 6/min, AVPU – Pain and a severe lesion will have KTS = 4+1+2+2=9.

Acute Physiology Score (APS)

APS is used in order to estimate APACHE II. APS = ∑(Variables) [30]. For example, a patient with rectal temperature of 40°C, systolic blood pressure of 140 mm Hg, heart rate of 90/min, 13 respirations/min, FiO_2 = 0.3 and PaO_2 = 81, the arterial pH of 7.40, sodium concentration in serum of 156 mmol/l, potassium concentration in serum of 6.5 mmol/l, creatinine level in serum of 3.6 mg/100 ml, hematocrit of 55%, 43 leucocytes/mm^3 GCS = 8 and bicarbonate ion concentration of 33 mmol/l will have an APS = 3 + 3 + 0 + 0 + 0 + 0 + 0 + 2 + 3 + 4 + 2 + 4 + (15-8) + 1 = 29.

Simplified Acute Physiology Score (SAPS)

The algorithm derived from APS (Acute Physiology Score) is used to determine the severity of a pathological condition, not necessarily for a traumatism, the parameters that are taken into consideration are: age, heart rate, systolic blood pressure, temperature, GCS, mechanical ventilation, PaO_2, FiO_2, urine output, level of blood urea, plasmatic concentrations of sodium, potassium, bicarbonate, bilirubin, leucocytes, chronic pathological conditions, each one of them has a specific score and is calculated using a specific formula [31]. The most recent model is the 3d model (SAPS III), the points that are given for each of the criteria are listed below: age (years): <40 (0 pts); ≥40 and <60 (5 pts); ≥60 and <70 (0 pts); ≥70 and <75 (13 pts); ≥75 and <80 years (15 pts); ≥80 (18 pts); comorbidities: cancer therapy (3 pts); Chron's disease, cardiopathies, hematologic cancer (6 pts); cirrhosis, AIDS (8 pts); metastatic cancer (11 pts). Length of stay before ICU admission period (days): <14 (0 pts); ≥14 and <28 (6 pts); ≥28 (7 pts). Intrahospital location before ICU admission: emergency room (5 pts), another ICU (7 pts), another department (8 pts). Use of major therapeutic options before ICU admission: vasoactive drugs (3 pts). ICU admission: unplanned (3 pts), planned (0 pts); reason for ICU admission: rhythm disturbances (-5 pts); seizures (-4 pts); hypovolemic hemorrhagic shock, hypovolemic non-hemorrhagic shock, digestive tract pathological conditions (acute abdomen for example) (3 pts); coma, stupor, obtunded patient, vigilance disturbances, confusion, agitation, delirium (4 pts); septic shock, anaphylactic shock, mixed and undefined shock (5 pts); liver failure (6 pts); focal neurological deficit (7 pts); severe pancreatitis (9 pts); intracranial mass effect (10 pts); surgical status at ICU admission: scheduled surgery (0 pts); no surgery (5 pts); emergency surgery (6 pts). Anatomical site of surgery: transplantation surgery (liver, kidney, pancreas etc.) (-11 pts); trauma – isolated, multiple (-8 pts); cardiac surgery (-6 pts); neurosurgery (5 pts). Acute infection at ICU admission – nosocomial (4 pts); respiratory (5 pts). GCS: 3-4 (15 pts); 5 (10 pts); 6 (7 pts); 7-12 (2 pts); ≥13 (0 pts). Total bilirubin – highest (in mg/dl): <2 mg/dl (0 pts); ≥2 and <6 mg/dl (4 pts); ≥6 mg/dl (5 pts). Total bilirubin – highest (in μmol/l): <34.2 μmol/l (0 pts); ≥34.2 and <102.6 μmol/l (4 pts); ≥102.6 μmol/l (5 pts). Body temperature – highest (in °C): <35 °C (7 pts); ≥35 °C (0 pts). Creatinine – highest (in mg/dl): <1.2 mg/dl (0 pts); ≥1.2 mg/dl and <2 mg/dl (2 pts); ≥2 and <3.5 mg/dl (7 pts); ≥3.5 mg/dl (8 pts). Creatinine – highest (in μmol/l): 3-4 μmol/l (15 pts); 5 μmol/l (10 pts); 6 μmol/l (7 pts) <106.1 μmol/l (0 pts); ≥106.1 and <176.8 μmol/l (2 pts); ≥176.8 and <309.4 μmol/l (7 pts); ≥309.4 μmol/l (8 pts). Heart rate: <120 /min (0 pts); ≥120 and <160 /min (5 pts); ≥160 /min (7 pts). Leucocytes – highest: <15 g/l (0 pts); ≥15 g/l (2 pts). Hydrogen ion concentration – lowest: ≤7.25 (3 pts); >7.25 (0 pts). Platelets – lowest: <20 g/l (13 pts); ≥20 and <50 g/l (8 pts); ≥50 and <100 g/l (5 pts); ≥100 g/l (0 pts). Systolic blood pressure – lowest: <40 mm Hg (11 pts); ≥40 and <70 mm Hg (8 pts); ≥70 and <120 mm Hg (3 pts); ≥120 mm Hg (0 pts). Oxygenation: PaO_2/FiO_2 <100 and VM (11 pts); PaO_2/FiO_2 ≥100 and MV (7 pts); PaO_2<60 without MV (5 pts); PaO_2≥60 without MV (0 pts).

Afterwards, we calculate SAPS III. To include it in the general formula for chance of survival, we must first calculate b = -32.6659 + ln(SAPS III + 20.5958) x 7.3068, and then P(survival) = $e^b/(1+e^b)$ [32].

For example, a 33-year old patient, without comorbidities, was admitted for 5 days, the admission in ICU was planned, the reason for admission – seizures, he had undergone an emergency surgery of liver transplantation, without acute infections, GCS = 8, bilirubin – 5 mg/dl, creatinine – 6 μmol/l, heart beats – 170/min, leucocytes highest level – 13 g/l, lowest blood pH level – 7.23, lowest platelets level of 19 g/l, minimum systolic blood pressure of 73 mm Hg, PaO_2 = 61 without need of intubation. The patient will have SAPS = 0 + 0 + 0 + 0 – 4 + 6 – 11 + 0 + 2 + 4 + 8 + 7 + 0 + 3 + 13 + 3 + 0 = 31, after that b = -32.6659 + ln(31+

+ 20.5958) x 7.3068 = -3.85197, and then we introduce it in the general formula P(survival) = $e^{-3.85197}/(1+e^{-3.85197})$ = 0.0207, in this case the chance for survival is approximately 2.07%.

Therapeutic Intervention Scoring System (TISS)

TISS is useful in assessing the best treatment strategy for the patients that are admitted in the ICU. Its criteria are grouped into 4 categories (each of them is contributing with 1, 2, 3 and respectively 4 points to the overall score in the 1983 version).

After we calculate TISS = ∑(Conditions) we can classify the patients in 4 categories – Classs IV (≥40 points); Class III (20-39 points); Class II (10-19 points); Class I (<10 points). Class III and Class IV patients require an experienced nurse, Class III patients that are relatively stable can be placed together with Class II patients, a nurse can take care of 4 Class II patients, Class I patients do not require admission in the ICU and observation, except the cases when there is present a myocardial infarction [33]. For example, a patient with peritoneal dialysis (4 points), that requires platelet transfusion (4 points) and blind intratracheal suctioning (3 points) will have TISS = 4 + 4 + 3 = 11 and will be categorized as a Class II patient.

Sequential Organ Failure Assessment score (SOFA) and qSOFA (quick-SOFA)

SOFA is used to determine the number and severity (quantity and quality) of a multi-organ disfunction, the criteria used for this score are: PaO_2/FiO_2, platelets level, GCS, bilirubin level, systolic blood pressure, creatinine level and the urine output. SOFA = ∑(Variables).

Also, SOFA can be used to predict the chance of death. In condition if ΔSOFA ≥ 2, the patient has a chance of survival two times less and when ΔSOFA ≤ -2 the same patient has a double chance of survival. Jones A. et al. reported that ΔSOFA ≥ 2 (42% chance of death); ΔSOFA =1 (23% chance of death); ΔSOFA = 0 (19% chance of death); ΔSOFA = -1 (11% chance of death); ΔSOFA ≤ -2 (9% chance of death) [34]. For example, a patient with PaO_2/FiO_2 of 50, SaO_2/FiO_2 of 253, blood platelets level of 140 x $10^3/mm^3$, bilirubin level of 1.5 mg/dl, systolic blood pressure of 90 mm Hg and the dopamine level of 4 pg/ml, GCS = 13, creatinine level of 1.3 mg/dl, urine output 400 ml/d will have $SOFA_1$ = 4 +1 + 1 + 1 + (0+2) + 1 + 1 + 3 = 11, afterwards the value of PaO_2/FiO_2 modified to 250, respectively $SOFA_2$ = 2 + 1 + 1 + 1 + (0+2) + 1 + 1 +3 = 9, respectively ΔSOFA= $SOFA_2$ - $SOFA_1$ = 9 – 11 = -2, the chances of death for this patient are estimated to approximately 9%.

The qSOFA is the simplified version of SOFA, it contains only 3 clinical criteria that can be very easily appreciated: A – Altered mental status (GCS < 15); R – Respiratory rate (≥ 22 respirations/minute) and S – Systolic blood pressure (≤ 100 mm Hg). It is calculated using the formula:

qSOFA = A + R + S, the criteria listed above are binary. If the expressed conditions are true, then they are equal with 1. In condition if qSOFA ≥ 2, then there is a high change of poor outcome [35]. For example, a patient with GCS = 13; respiratory rate of 25 respirations/min and systolic blood pressure of 63 mm Hg will have a qSOFA = 1 + 1 +1 = 3.

Acute Physiology, Age, Chronic Health Evaluation II (APACHE II)

APACHE II is a score used to determine the severity of a pathological condition, it is derived from the following criteria: APS, AS and PSI (APS – Acute Physiology Score; AS – Age Score; PSI – Points for surgical interventions), the points that are given in this case are:

APS – the exact value (see above); age (AS): ≤44 (0 pts); 45-54 (2 pts); 55-64 (3 pts); 65-74 (5 pts); ≥75 (6 pts). Points for surgical interventions (PSI): for nonsurgical or postoperative patients that had undergone an emergency surgery (5 pts); for postoperative patients that had undergone a planned surgery (2 pts). APACHE II = APS + SV + PIC, b = -3.517 + (0.146 x APACHEII) + 0.603 x S + Y [30]. Where S is a binary value equal to 1 when the patient has undergone an emergency surgery, Y is a constant that is attached to chronic diseases. Afterwards b is introduced in the formula P(survival) = $e^b/(1+e^b)$ [36]. As an example, we will take a patient with APS = 29, 33 years old, with multiple traumas and emergency surgical intervention (y = -1.081). APACHE II = 29 + 0 – 5 = 24, x = -3.517 + (0.146 x 24) + 0.603 x 1 – 1.081 = -0.491, and then we calculate P(survival) = $e^{-0.491}/(1+e^{-0.491})$ = 0.3796, in this case the chance for survival is equal to approximately 37.96%.

Revised Trauma Score (RTS)

RTS has inversely proportional value with the severity of trauma, it is calculated using the following model: RTS = b_0 + b_1 x GCS + b_3 x SBP + b_3 x RR, where SBP – systolic blood pressure; RR – respiration rate, the constants – b_0= -3.5718; b_1 = 0.9368; b_2 = 0.7326; b_3= 0.2908. After that, the chances for survival are calculated using the standard formula P(survival) = $e^b/(1+e^b)$ if we consider that RTS = b [37]. For example, a patient with GCS = 7, systolic blood pressure of 120 mm Hg, and the respiration rate of 6/min has RTS = -3.5718 + 0.9368 x 2 + 0.7326 x 4 + 0.2906 x 2 = 1.8134, P(survival) = $e^{0.8597}/(1+e^{0.8597})$ = 0.8597, respectively in this case the patient has a chance of survival equal to approximately 85.97%.

Triage RTS (T-RTS)

T-RTS is used to assess the dynamics in the state of a trauma patient using the same criteria as RTS, but in the case of T-RTS we calculate the Δ values in order to appreciate how the patient's state has changed according to the following formula: ΔT-RTS = $TRTS_{at\ hospital}$ – $TRTS_{on\ scene}$.

There are three variants – ΔT-RTS = 0 (No Change); ΔT-RTS ≥ 1 (Improving); ΔT-RTS < 0 (Deteriorating) [38]. For example, a patient with a respiratory rate of 23 respirations/min, systolic blood pressure of 63 mm Hg and GCS = 12 will have a $TRTS_{on\ scene}$ = 4 + 2 + 3 = 9, systolic blood pressure raised at 79 mm Hg and GCS = 13 in hospital, $TRTS_{at\ hospital}$ = 4 + 3 + 4 = 11, ΔT-RTS = 11 – 9 = 2, we conclude that this patient's state is improving, whereas his initial state was poor.

Glasgow Coma Score (GCS)

This is a score widely used by neurologists, neurosurgeons, anesthesiologist etc. It takes into consideration eye

opening, verbal response and motor response. The value can be directly proportional to the patient's consciousness. The spontaneous eye opening is characterized by opening without stimulating the patient, the patient is doing it consciously, the eye opening on verbal stimulus is when the patient is opening his eyes when is verbally called, the eye opening on pain stimulus is when the patient is opening his eyes after causing him physical pain sensations, and not reacting on verbal stimuli. The oriented verbal response is the response given by an auto- and allopsychicaly oriented patient, the confused verbal response is a logic bond order of words that cannot be understood, with the patient being oriented on the circumstances, the abstract verbal response is a verbal response that doesn't have a logical continuity and is not oriented on the circumstances, the incomprehensible verbal response is a continuity of words that cannot be understood. The obeying motor response is the response in which the patient is following the doctor's commands, the pain localized motor response is the response in which the patient is trying to palpate the topographical region in which the pain is localized, the avoiding pain motor response is the response in which the patient is avoiding the topographical region in which the pain is localized, the abnormal flexion motor response is the response in which a body part is flexed spontaneously and usually accompanied by abduction, the abnormal extension motor response is the response in which the patient has a body part that is spontaneously extending and it is usually accompanied by adduction.

A patient that is opening eyes on a pain stimulus, has confused verbal response and pain localized, his motor response will have a GCS = 2 + 4 + 5 = 11. After a craniocerebral trauma, in the case of GCS = 13-15, we can suspect a mild traumatic brain injury, in cases when GCS = 9-12, we can suspect a moderate traumatic brain injury, in cases when GCS = 3-8, we can suspect a severe traumatic brain injury, some researchers say that severe traumatic brain injuries do not correspond to GCS = 8 but GCS <8 [39].

Mixed scores

The mixed scores combine the anatomical, functional and other criteria, most of them there described above:

Mortality Probability Admission Model (MPMoIII)

This is a score used to determine the chances of survival based on the following criteria: physiological parameters, acute and chronic diagnoses, mechanical ventilation, reason for admission in ICU, age and other details that will be discussed afterwards [51]. The most recent model that we can use is MPMoIII, which can be calculated the following way: MPMoIII = $b_0 + b_1 + b_3 \times Age + b_4 + b_5 + b_6 \times \alpha + b_7$ where b_0 – general constant; b_2 – comorbidity constant; b_3 – age constant; b_4 – constant for other situations; b_5 – constant to identify the cardiac and respiratory arrest; $b_6 \times \alpha$ – the interaction product between 2 factors; b_7 – the physiological constant. Each of these constants has certain values that can be associated with the patient. To understand how this score must be calculated, we must take into consideration that constants in the same category can be simultaneously in the formula. For example, a patient with metastatic neoplasm and cirrhosis will have both constants included in the formula, the factor interactions can be introduced in the formula only when the main factor is present and then the constant is multiplied to the age, afterwards to calculate the score we consider MPMoIII = b and introduce it in the standard formula P(survival) = $e^b/(1 + e^b)$ [40].

A patient that is 56-year old, GCS = 4, heartbeats = 161/min, metastatic neoplasm, gastrointestinal bleeding, in which a respiratory arrest was identified, was resuscitated during the ICU stay and needed an unplanned surgical intervention will have MPMoIII = -5.36283 + 2.050514 + 0.433188 + 3.204902 – 0.165253 + (53x0.0385582) – 0.7969783 – (53 x 0.0330237) = -0.3431288, and P(survival) = $e^{-0.3431288}/(1 + e^{-0.3431288})$ = 0.4150496549, respectively the chance for survival in this case is equal to approximately 41.50%.

Harborview Assessment for Risk of Mortality (HARM)

HARM is a mixed score developed based on the ICD-9-CM and takes into consideration the mechanism of injury, anatomical criteria, comorbidities and age. It is calculated using the formula $b = b_0 + b_1 + \ldots + b_n(\alpha \times \beta)$ where the b_1, b_2,…, b_n are constants that are attached to different conditions, the α x β product is an interaction product that will be included in the formula only in the case when both of the product criteria are present. For example, the variable *head x spinal cord* will be added when both of these anatomical structures had undergone a lesion. In the case of age constants, we multiply them by the patient's age expressed in years. According to the results obtained by West T.A., HARM manifested a better performance compared to TRISS and ICISS. Also, it is important to mention comorbidity constants: congenital coagulopathy (1.494934), cirrhosis (2.954898), ischemic heart disease (0.9844608), hypertension (- 0.546734), psychoses (-1.854641) and alcohol or drug dependence (-0.7681033), after calculating the b value, if we consider HARM = b, then we can introduce this value in the standard formula P(survival) = $e^b/(1 + e^b)$ [41]. For example, a 46-year old patient has cirrhosis and had undergone a skull fracture with incomplete spinal cord injury above the C4 segment. He has b = -4.708587 - 0.2163938 × 46 + 0.0109741 × 46 + 0.0019716 × 46 + 2.954898 + 0.6120652 + 1.879599 + 0.7507725 = 1.8678561, and respectively P (survival) = $e^{1.8678561}/(1 + e^{1.8678561})$ = 0.8662, respectively, in this case, the chances for survival are equal to approximately 86.62%.

Trauma and Injury Severity Score (TRISS)

TRISS is a score used to predict the consequences of a trauma. It is derived from RTS and ISS, ISS is calculated using the usual formula, and then we calculate $b = b_0 + b_1 \times RTS + b_2 \times ISS + b_3 \times AgeConst$, where $b_{0 =} -1.29803310$, $b_{1 =}$ 0.89538700, $b_{2 =} -0.09521947$ and $b_{3 =} -1.27540759$ in case of penetrating traumas and $b_{0 =} -1.64790049$, $b_{1 =}$ 0.90535734, $b_{2 =} -0.07845091$, $b_{3 =} -1.38013670$ for blunt traumas , where AgeConst is a binary value (0 if Age < 55, 1 if Age ≥ 55). The chance of survival can be further calculated using the standard formula P(survival) = $e^b/(1 + e^b)$ [42]. For example, a patient with RTS = 1.8134,

ISS = 16, penetrant traumas, age of 31 years will have TRISS = -1.29803310 + 0.89538700 × 1.8134 – 0.09521947 × 16 + 0 = -1.1978498342, after that P (survival) = $e^{-1.1978498342}/(1 + e^{-1.1978498342})$ = 0.2318579399, the chance for survival in this case is approximately 23.18%.

New Trauma and Injury Severity Score (NTRISS)

This score is a NISS, RTS and GCS derived scale. It is calculated using the $b = b_0 + b_1 \times MR + b_2 \times SBP + b_3 \times NISS + b_4 \times AgeConst$, where SBP – systolic blood pressure in RTS, MR – motor response in GCS, AgeConst is a binary value (0 if Age < 55, 1 if Age ≥ 55), its constants are – for penetrating traumas – $b_{0 =} -1.58632944$, $b_{1 =} 0.58883203$, $b_{2 =} 0.96952677$, $b_{3 =} -0.06659814$, $= -1.00582810$, for blunt traumas – $b_{0=} -1.67602650$, $b_1 = 0.61944706$, $b_{2=} 0.89539814$, $b_{3=} -0.07289039$, $b_4 = -1.33088941$, then, the b value is introduced in the standard formula P(survival) = $e^b/(1 + e^b)$ [42]. For example, a 57-year old patient with MR = 4 in GCS, SBP = 2 in the RTS scale; NISS = 27 and blunt traumas has a NTRISS = -1.67602650 + 0.61944706 × 4 + 0.8953814 × 2 – 0.07289039 × 27 – 1.33088941 × 1 = -0.7064054, afterwards P(survival) = $e^{-0.7064054}/(1 + e^{-0.7064054})$ = 0.3303936013, in this case, the chances for survival are equal to approximately 33.03%.

Trauma and Injury Severity Score with SpO_2 (TRISS SpO_2)

It is a recently developed score that takes into consideration the SpO_2 (Peripheric oxygen saturation). It is calculated using the following model – $b = b_0 + b_1$ x GCS + b_2 x SBP + b_3 x SpO_2 + b_4 x ISS + b_5 x AgeConst, where GCS – Glasgow Coma Scale, points being accorded from RTS, SBP – systolic blood pressure calculated based on RTS, ISS – Injury Severity Score, AgeConst is a binary value (0 if Age < 55, 1 if Age ≥ 55), SpO_2(0 if it can't be measured; 1-80% = 1; 81-90% =2; 91-95% = 3; 96-100% = 4), the constants for this score are: in case of penetrant trauma – $b_0 = -3.5166820$, $b_1 = 0.8515884$, $b_2 = 0.3453793$, $b_3 = 1.3098071$, $b4 = -0.1955984$, $b_5 = -4.0353761$, in case of blunt trauma – $b_0 = -2.97523446$, $b_1 = 0.75773826$, $b_2 = 0.58321377$, $b_3 = 0.38492625$, $b_4 = 0.08441861$, $b_5 = -1.59455370$, after that, the chances for survival are calculated using the following formula: P (survival) = $e^b/(1 + e^b)$ [42]. For example, a 43-year old patient with GCS – 3 points in RTS scale, SBP = 2 points in RTS scale, SpO_2 = 3 points, ISS = 18 and blunt trauma will have TRISS SpO_2 = -2.97523446 + 0.75773826 x 3 + 0.58321377 x 2 + 0.38492625 x 3 + 0.08441861 x 18 + 0 = 2.36886909, and then P(survival) = $e^{2.36886909}/(1 + e^{2.36886909})$ = 0.9144224937, respectively, in this case, the chances for survival will be approximately 91.44%.

New Trauma and Injury Severity Score with SpO_2(NTRISS SpO)

NTRISS $SpO_2$is also a recently developed scale that takes into consideration the peripheric oxygen saturation. It is calculated using the following formula – $b = b_0 + b_1 \times MR + b_2 \times SBP + b_3 \times SpO_2 + b_4 \times NISS + b_5$ x AgeConst, where MR – motor response points according to GCS, SBP – systolic blood pressure according to RTS, AgeConst is a binary value (0 if Age < 55, 1 if Age ≥ 55), SpO_2 (0 if it can't be measured; 1-80% = 1; 81-90% =2; 91-95% = 3; 96-100% = 4), the constants are: in case of penetrating trauma – $b_0 = -1.5156694$, $b_1 = 0.1832071$, $b_2 = 1.0209288$, $b_3 = 1.1288631$, $b_4 = -0.1138697$, $b_5 = -1.7286860$, in case of blunt traumas – $b_0 = -2.73634921$, $b_1 = 0.59396868$, $b_2 = 0.66226833$, $b_3 = 0.56405908$, $b_4 = -0.06841853$, $b_5 = -1.43274160$, afterwards, the chance of survival is calculated using the standard formula P(survival) = $e^b/(1 + e^b)$ [42]. For example, a 56-year old patient with MR = 3 points according to GCS, SBP = 3 points according to RTS, SpO_2 = 2 points, NISS = 31, and penetrating trauma will have NTRISS SpO_2 = -1.5156694 + 0.1832071 × 3 + 1.0209288 x 3 + 1.1288631 x 2 – 0.1138697 × 31 -1.7286860 × 1 = -0.9041822, then P(survival) = $e^{-0.9041822}/(1 + e^{-0.9041822})$ = 0.2881918128, respectively, patient's survival chance is approximately 28.81%.

A Severity Characterization of Trauma (ASCOT)

ASCOT takes into account – AgeConst which is a binary value (0 if Age < 55, 1 if Age ≥ 55), GCS value, systolic blood pressure and respiration rate based on RTS, ISS calculated based on AIS85, which is more effective in the opinion of the authors, similarly with TRISS, it has specific constants that take into consideration the mechanism of trauma: in case of penetrant trauma – b_0 = -1.1350, b_1 = 1.0626, b_2 = 0.3638, b_3 = 0.3332, b_4 = -0.3702, b_5 = -0.2053, b_6 = -0.3188, b_7 = 0.8365, in case of blunt trauma – b_0 = -1.1570, b_1 = 0.7705, b_2 = 0.6583, b_3 = 0.2810, b_4 = -0.3002, b_5 = -0.1961, b_6 = -0.2086, b_7 = -0.6355 [43], the variables considered are part of APC (Anatomical Profile Characterization) – A (severe traumas with AIS ≥ 3 in the head region, brain and spinal column), B (thorax and the anterior portion of neck), C (severe traumas in other body regions) and D (lesions with AIS = 1 and 2 that are present in any body region), they are further included in the following formula $b = b_0 + b_1 \times$ GCS + b_2 x SBP + b_3 x RR + $b_4 \times$ A + $b_5 \times$ B + b_6 x C + $b_7 \times$ AgeConst, where SBP – systolic blood pressure according to RTS, GCS – Glasgow Coma Score and RR – respiratory rate according to RTS, AgeConst – The age constant. The survival chances are then appreciated using the standard formula P(survival) = $e^b/(1 + e^b)$ [44]. For example, a 34-year old patient with a blunt trauma GCS = 3 points, SBP = 2 points according to RTS; RR = 3 points according to RTS, $AIS_{Head\ and\ neck}$ = 3; AIS_{Thorax} = 4; $AIS_{Lower\ limb}$ = 4 will have an ASCOT = -1.1570 + 0.7705 × 3 + 0.6583 × 2 + 0.2810 × 3 -0.3002 ×x 3 – 0.1961 × 4 - 0.2086 × 4 + 0 = 0.7947, and then P(survival) = $e^{0.7947}/(1 + e^{0.7947})$ = 0.6888396197, respectively, in this case, survival chances are equal to approximately 68.83%. We should mention that, in case of ASCOT, TRISS and NTRISS, the mechanism of trauma is taken into consideration (blunt or penetrating) [43].

Revised Injury Severity Classification (RISC)

RISC is used to evaluate the survival chances considering the following variables: age, NISS, AIS for head, AIS for extremities, GCS, thromboplastin action time, base excess, preclinical cardiac arrest presence, preclinical systolic blood pressure, the most recent version of this score is the II edition [45]. After that, we calculate it in the following way –

the initial variable is equal to 5, we then add the points from the table depending on the patient's condition, RISC = 5.0 + ∑(Variables), and then we consider RISC = b, and introduce it in the standard formula P(survival) = $e^b/(1 + e^b)$ [46]. For example, a 40-year old patient with NISS = 27, = 0; = 5; GCS = 12; TT = 45 s; without base excess, SBP = 85 mm Hg, without cardiac arrest will have a RISC = 5.0 – 0 – 0.03 x 27 – 0 – 1 – 0 – 0.8 – 0 – 0.4 – 0 = 1.99, and then P(survival) = $e^{1.99}/(1 + e^{1.99})$ = 0.8797431375, respectively, this patient has an approximately survival chance equal to 87.97%.

Pediatric Trauma Score (PTS)

This is a score used exclusively for pediatric patients. It includes the following variables: body weight, airway status, systolic blood pressure, central nervous system status, skeletal traumas, skin lesions.

We differentiate 5 groups of risk in this case, for PTS = 9-12, the death risk is equal to 0%, for PTS = 7-8, the death risk is equal to 3%, for PTS = 5-6, the death risk is equal to 15%, for PTS = 3-4, the death risk is equal to 36%, for PTS = 1-2, the death risk is equal to 45%, for PTS ≤ 0, the death risk is equal to 100% according to the original article [47]. For example, a patient with a body weight of 15 kg, normal airway status, systolic blood pressure of 80 mm Hg, alert CNS status, a closed fracture and without skin lesions will have a PTS = 1+2+1+2+1+2=9 and 0% risk of death.

Norwegian Prediction Model in Trauma 2 (NORMIT2)

It was developed in Norway. Its coefficients are derived from AIS98. NORMIT2 can be calculated using T-RTS, NISS, age and ASA-PS attached coefficients by introducing them in the following formula: NORMIT2 = (0.5562 x T-RTS) – 4.3234 x $[(Age + 1)/100]^3$+ ASA, where ASA is the individual American Society of Anesthesiologists physical status classification system (ASA-PS) categories estimated before the injury (ASA1 = (-0.0713 x NISS) + 0.6266, ASA2 = (-0.0565 x NISS) – 0.2142, ASA3 = (-0.0487 x NISS) – 0.8971, ASA4 = (-0.0081 x NISS) – 3.8748). The result of NORMIT2 score is considered as coefficient *b* in standard logistic regression equation - P(survival) = $e^b/(1 + e^b)$ [48]. For example, a 41-year old ASA1patient with a T-RTS = 3 and NISS = 31 will have a $NORMIT^2$ = (0.5562 x 3) – 4.3234 x $[(41+1)/100]^3$ + (-0.0713 x 31) + 0.6266 = -0.213073, afterwards P (survival) = $e^{-0.213073}/(1 + e^{-0.213073})$ = 0.4469, the chances for survival are equal to 44.69% in this case.

Trauma Audit and Research Network (TARN) Probability of survival model

This model was developed in 2019 and is constantly updated by the Trauma Audit and Research Network using its own institutional database of trauma patients. It takes into consideration the following criteria: ISS, GCS, modified Charlson Comorbidity Index (mCCI), age, gender, intubation necessity and the interactions between these factors using the following formula *b* = GenConst AgeVar + GenderVar + √(10/ISS) – 0.8618 + $\log_e$(ISS/10) – 0.2974 + GCS_{var} + $mCCI_{var}$ + Interactions, where GenConst – general constant, Age_{var} – age variable + $Gender_{var}$ – gender variable, ISS – Injury Severity Score, $mCCI_{var}$ – modified Charlson Comorbidity Index. Afterwards we use the formula: P(survival) = $e^b/(1 + e^b)$ [49].

The Sequential Trauma Score (STS)

STS is a scale that takes into consideration the patient's data (P), preclinical measured physiological variables (A), early clinical physiological variables (B1) and late clinical physiological variables (B2) of a traumatic patient. The coefficients for equation may vary for different regions where the score was validated. The survival chance is calculated depending on the information that is available at the 4 different periods of contact with the patient:

For Model P – P (survival) = 1 - (1 / (1 + EXP (2.268 - 1.234 x AgeConst))).

For Model P+A – P (survival) = 1 - (1 / (1 + EXP (3.566 - 1.653 x AgeConst - 1.353 x GCS - 1.311 x PreclinicalAnisocoria – 0.983 x SBP - 0.78 x HR))).

For Model P+A+B1 – P (survival) = 1 - (1 / (1 + EXP (3.901 - 1.663 x AgeConst - 0.602 x SBP - 0.7 x PreclinicalAnisocoria - 1.11 x GCS - 1.294 x ClinicalAnisocoria - 1.316 x BE - 0.756 x SpO_2 - 0.947 x TT))).

For Model P+A+B1+B2 – P (survival) = 1 - (1 / (1 + EXP (4.857 - 1.333 x CCCC - 0.772 x MT - 0.345 x MAIS (=4) - 2.199 x MAIS (=5) - 1.73 x AgeConst - 0.752 x GCS - 0.647 x PreclinicalAnisocoria - 1.251 x ClinicalAnisocoria - 0.98 x BE - 0.711 x TT))).

Where AgeConst – age constant; GCS – Glasgow Coma Scale; SBP – systolic blood pressure; HR – heart rate; BE – base excess; TT – Thromboplastin action time; CCCC – closed chest cardiac compressions; MT – massive transfusion; MAIS – maximum AIS. In the model P+A+B1+B2, MAIS is considered only in the cases when it is equal to 4 or 5, in case when it is equal to 4, only one of the variables listed above is considered, this means that the product -2.199 x MAIS (=5) is not included in the formula. The authors of the original article note that this score is not efficient to evaluate blunt trauma patients [50].

As an example, we will review a 62-year old patient at the late clinical period – no necessity for CCCC, necessity for massive transfusion, MAIS = 4, GCS = 13, without clinical or preclinical anisocoria, base excess = -9, thromboplastin action time reduced by 61%, respectively P(survival) = 1 – (1/(1+EXP(4.857 – 1.333 x 0 – 0.772 x 1 – 0.345 x 4 x (-4) – 2.199 x 0 – 1.73 x 1 – 0.752 x 0 – 0.647 x 0 – 1.251 x 0 – 0.98 x 1 – 0.711 x 1))) = 0.3282744176, respectively, in this case, the chances for survival are equal to approximately 32.82%.

World Dispersion

Europe

In Germany, the AIS, ISS, NISS, GCS and RISCII in association with wbCT (whole body computer tomography) are used [51, 52, 53, 54, 55]. In France, T-RTS, TRISS, MGAP are widely used [56, 57].The Scandinavian countries widely exploit the ISS [58]. In Norway, TRISS, TARN and NORMIT2 are introduced in daily practice. Taking into account that NORMIT 2 was developed in Norway's population, it has the best characteristics to predict the outcomes in local medical system. TARN was proposed by UK scientific team [48]. For Spain population, the implementation of ASCOT,

ICISS and TRISS was discussed [59]. APACHE II, RTS and GCS are widely used in Romania without any validation [60]. ASCOT or TRISS were compared by the Bucharest clinicians with no significant difference in their usage for the Romanian population [61].

Asia and Oceania

In China, there are implemented the ISS, TRISS, RTS algorithms and to appreciate the severity of traumas, instead of wbCT, the ultrasonography is recommended [62]. NISS methodology wasn't accepted, because a study has proven that it has similar efficiency with ISS in this zone. Also, this study proves that ISS74 and ISS97 have a similar accuracy [2]. LISS score was proposed for the first time in Hangzhou in 2012 [18]. In Korea, doctors consider the RTS and value of the serum albumin [63]. In Australia, AIS, ISS and TRISS methodology is accepted [64, 65, 66]. Taiwan's medical system benefits by GCS, AIS, ISS and RTS scores [7, 67].

South America

The clinicians from Brazil have developed a new score – NTRISS, that shows similar performance with TRISS [42]. Also in Brazil, the independent diagnostic criteria as peripheral oxygen saturation, lactate concentration, GCS, infused crystalloid volume, presence of TBI (Traumatic Brain Injury) are considered in patient's trauma assessment [69]. In Colombia, the ISS, RTS and TRISS are used for severity trauma characterization [68].

North America

With no matter that TRISS is originary from USA, there is used a series of scales in order to evaluate the severity of trauma as follows: ISS, NISS, TRISS, ICISS MGAP, GAP HARM and KTS [41, 72, 73, 74].

Republic of Moldova

In the Republic of Moldova, there have been used TS, RTS, AIS and ISS scores. The majority of traumatic scores haven't statistical validation [75]. We also have identified that in the ICU, the APACHE II score was used by the anesthesiologists to predict mortality rate for critical patients without any validation as well [76]. For the patients with associated trauma and TBI (Traumatic Brain Injury), the clinicians from the Republic of Moldova use MGAP and ASCOT, because they include the SBP (Systolic Blood Pressure) and GCS (Glasgow Coma Score). In the opinion of these authors, SBP and GCS are good predictors of survival in case of TBI, but there is no evidence that these scores are able to predict the evolution of trauma patients better than other scores mentioned before [77].

At present, there is only one validated trauma score – NISS. Also, MPMoIII was used in a pilot research with relatively reduced number of respondents and the accuracy of coefficient needs improvement. The ability of NISS to predict the probability of survival rate was estimated in the retrospective study that enrolled 467 severe trauma patients and 225 critical trauma patients admitted in Emergency Medicine Institute (EMI). The modelling for critical trauma patients had a good fit in comparison with severe trauma patients [16]. Both, the MPMoIII or/and NISS were tested for survival prediction in severe trauma patients (NISS>15) transferred from regional hospitals to EMI. According to these results, the NISS has a better prediction power than MPMoIII (Nagelkerke R square was 64.1% vs 51%, mixed model having it equal to 81%). In comparison with patients admitted directly in EMI, the determination coefficient increased by more than 20% (40% and 64.1%, respectively) [17], being a serious argument to continue studies in this direction.

Conclusions

Different traumatic scores are used worldwide (different continents, countries or regions) to estimate the severity of trauma patients in relation to the anatomical, physiological or combined criteria. All of them have a potential to be validated for the Moldovan medical system.

In perspective, a part of these scores will be validated and compared to identify those ones that have the best determination, calibration and discrimination abilities to predict the outcomes for the local medical system. As a result, the coefficients from the mathematical equations belonging to the scores will be adjusted to the conditions of the national medical system of the Republic of Moldova.

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Authors' ORCID iDs and academic degrees
Oleg Arnaut – https://orcid.org/0000-0002-5483-8672, MD, PhD.
Dan Croitoru – https://orcid.org/0000-0002-8915-0157, MD.
Ion Grabovschi – https://orcid.org/0000-0002-7716-9926, MD.
Serghei Sandru – https://orcid.org/0000-0002-2973-9154, MD, PhD.

Authors' contributions
OA designed the trial and interpreted the data. DC drafted the first manuscript. IG interpreted the data. SS revised the manuscript critically. All the authors approved the final version of the manuscript.

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The monograph "Evolution of otitis media in children"

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The author: **Svetlana Diacova**, MD, PhD, Associate Professor, Department of Otorhinolaryngology
Nicolae Testemitsanu State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova

Reviewed by **Mihail Maniuc**, MD, PhD, Professor of Otorhinolaryngology

The monograph describes the development, evolution and outcomes of otitis media (OM) in children, the middle ear (ME) pathology, which affects 90% of children. Some of OM forms provoke intracranial complications, chronic suppurative OM, hearing loss and permanent disability. The detection and prognostics of those OM forms and intensive treatment including surgical one in small children prevent from the negative disease evolution.

OM represents the group of ME pathology, which begins in early childhood from asymptomatic hearing loss, manifested by short-duration acute pain, continues to prolonged forms (persistent and recurrent OM) and progresses to chronic forms in adolescence. The significance of OM for child's development and uncertain criteria for differential diagnosis of various OM forms cause a large variety of treatment modalities and methods. Some of them influence the clinical improvement, but long-term results do not correlate with initial positive changes. Another approach significantly reduces recurrence and complication rates, but increases the number of ear surgery at an early age and makes tympanostomy (TS) the most frequently surgery performed in some countries. In Moldova chronic OM, otogenic complications and chronic hearing loss rates are relatively high, that indicates the necessity of further OM researches. The importance of this study is evident: understanding of OM transformational mechanism is the basis for the elaboration of curative and preventive approaches.

In collaboration with the Society "Pediatricians due Monde" (France) and Mayo Foundation, Mayo Clinic (USA) the author conducted several projects in order to create a system of OM management in Moldova. Specific goal of OM management is ME physical and acoustical restoration and prevention of OM persistence and recurrence.

The author presented analysis of OM development and evolution in childhood and their changes under the different methods of treatment on the basis of noninvasive monitoring of the middle ear status in big cohorts of children. The complex of noninvasive diagnostic tools which were used for this research is completed by microbiological, immunological and radiological exams, monitoring of quality of life (QL) and general health characteristics (GH).

Having analyzed the results of monitoring and assessment of the physical, acoustical data, hearing, QL and GH indexes the author evaluated the feasibility of therapeutic activities conducted in OM in the world and proposed the System of management of OM in childhood in Moldova.

The System is based on ME monitoring in children with high score risk factors (RF) for specification of treatment including surgical intervention. The diagnostic algorithm formulated by the author highlights the conditions and diseases, which contribute to OM evolution and progression. Detailed analysis and monitoring of electroacoustical and electrophysiological data of every child from the risk group discover tendency to persistence and recurrence of the ME pathology. The author recommends complex of examinations and treatment for symptomatic differentiation and independent OM forms. Exploring advantages and disadvantages of surgical procedures for OM in childhood the author elaborated the modified tympanostomy (MTS), designed for better functional results and prevention of OM persistence or recurrence. Post-surgical otomicroscopical and electro-acoustical monitoring demonstrated a stable high score of patients' hearing, QL and GH indexes.

The monograph is presented on 160 pages and consists of introduction, 4 chapters and general conclusions. Every chapter is completed by relevant bibliographic index with total number of 256 references. In the introduction the author reviews the background and scientific significance of the problem, determines the main aim and formulates the tasks of the study, describes international and national projects which formed the basis of this research.

The general part of the monograph is composed of 4 chapters. In the first chapter the author characterizes contemporary definitions, discusses features and study results of OM epidemiology, RF, pathogenesis and etiology. The second chapter is combined of clinical classification and diagnostics of OM in childhood. In the 3rd chapter the author describes methods of treatment – classical and contemporary and presents principles of the OM management. The 4th chapter contains data of the natural evolution of OM in children, formation of adhesive and persistent OM and the influence of treatment modality on the evolution of ME pathology. Conclusions of the monograph summarize the most important basic tendency of OM evolution in children and principles of OM management in childhood. The results of the research are illustrated in the monograph by 29 figures, 6 tables and 5 schemas.

Conclusion: the monograph "Evolution of otitis media in children" by Doctor Diacova Svetlana is the result of an original research with a certain scientific value and practical significance. It is recommended to otorhinolaryngologists, pediatricians, family doctors, residents and students.

Authors' ORCID iDs and academic degrees
Svetlana Diacova – https://orcid.org/0000-0003-1224-6481, MD, PhD.
Mihail Maniuc – https://orcid.org/0000-0002-5340-5779, MD, PhD.

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Address of the Editorial Office

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